DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number: PCT/US2015/044396
International filing date: 08 August 2015 (08.08.2015)
Document type: Certified copy of priority document
Document details: Country/Office: US
Number: 62/135,110
Filing date: 18 March 2015 (18.03.2015)
Date of receipt at the International Bureau: 21 August 2015 (21.08.2015)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-bis)
August 20, 2015

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 62/135,110
FILING DATE: March 18, 2015
RELATED PCT APPLICATION NUMBER: PCT/US15/44396

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS US62/135,110

Certified by

[Signature]
Under Secretary of Commerce
for Intellectual Property
and Director of the United States Patent and Trademark Office
### PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

**Express Mail Label No.** N/A

<table>
<thead>
<tr>
<th>INVENTOR(S)</th>
<th>FAMILY NAME OR SURNAME</th>
<th>CITY AND EITHER STATE OR FOREIGN COUNTRY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kate</td>
<td>MONROE</td>
<td>Berkeley, CA</td>
</tr>
<tr>
<td>Tina</td>
<td>SCHAWBE</td>
<td>San Francisco, CA</td>
</tr>
<tr>
<td>Francesca</td>
<td>AVOGADRI-CONNORS</td>
<td>San Mateo, CA</td>
</tr>
<tr>
<td>Ilaria</td>
<td>TASSI</td>
<td>San Francisco, CA</td>
</tr>
<tr>
<td>Helen</td>
<td>LAM</td>
<td>Union City, CA</td>
</tr>
</tbody>
</table>

Additional inventors are being named on the 1 separately numbered sheets attached hereto.

**TITLE OF THE INVENTION (500 characters max):**

ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF

Direct all correspondence to:

**CORRESPONDENCE ADDRESS**

- The address corresponding to Customer Number: 20872

**ENCLOSED APPLICATION PARTS (check all that apply)**

- Application Data Sheet. See 37 CFR 1.76. – 8 pgs.
- Drawing(s) Number of Sheets 47
- Specification (e.g. description of the invention) Number of Pages 289

**Fees Due:** Filing Fee of $220 ($130 for small entity) $85 (200 for small entity) $100 (200 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(a).

**METHODOF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT**

- Applicant asserts small entity status. See 37 CFR 1.27.
- Applicant certifies micro entity status. See 37 CFR 1.29.
- A check or money order made payable to the Director of the United States Patent and Trademark Office is enclosed to cover the filing fee and application size fee (if applicable).
- Payment by credit card. Form PTO-2038 is attached.

- The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 03-1952.

**TOTAL FEE AMOUNT ($)** 930.00

**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

I hereby certify that this paper is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: March 18, 2015

Signature: /Lila Olsen/ (Lila Olsen)

sf-3517693
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

<table>
<thead>
<tr>
<th>X</th>
<th>No.</th>
</tr>
</thead>
</table>

Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:

Yes, the invention was made under a contract with an agency of the U.S. Government. The name of the U.S. Government agency and the Government contract number are:

---

**WARNING:**
Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

**SIGNATURE** /Roberto K. Rodriguez/

**DATE** March 18, 2015

**TYPED or PRINTED NAME** Roberto K. Rodriguez

**REGISTRATION NO.** 66,244

**TELEPHONE** (415) 268-7215

**DOCKET NUMBER** 735023000600

---

sf-3517693
<table>
<thead>
<tr>
<th>First Named Inventor</th>
<th>Kate MONROE</th>
<th>Docket Number</th>
<th>735023000600</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INVENTOR(S)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Given Name (first and middle if any)</td>
<td>Family or Surname</td>
<td>Residence (City and either State or Foreign Country)</td>
<td></td>
</tr>
<tr>
<td>Arnon</td>
<td>ROSENTHAL</td>
<td>Woodside, California</td>
<td></td>
</tr>
</tbody>
</table>
ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

[0001] This invention relates to anti-TREM2 antibodies and therapeutic uses of such antibodies.

BACKGROUND OF THE INVENTION

[0002] Triggering receptor expressed on myeloid cells-2 (TREM2) is an immunoglobulin-like receptor that is expressed primarily on myeloid lineage cells, such as macrophages, dendritic cells, monocytes, Langerhans cells of skin, Kupffer cells, osteoclasts, and microglia; and is required for modulation of Toll-like receptor (TLR) signaling, the modulation of inflammatory cytokines, as well as for normal osteoclast development. TREM2 was discovered as a member of the TREM transmembrane glycoproteins, which belong to the single immunoglobulin variable (IgV) domain receptor family. The genes encoding human and mouse TREMs map to human chromosome 6p21.1 and mouse chromosome 17C3, respectively. The TREM cluster includes genes encoding TREM1, TREM2, TREM4, and TREM5, as well as the TREM-like genes in both human and mouse. Additionally TREM3 and plasmacytoid dendritic cell (pDC)-TREM were identified in mouse. The TREM-like genes, TREML1 and TREML2 in humans, and Trem1 and Trem12 in mouse, encode TLT-1 and TLT-2 proteins respectively. The two best characterized of these receptor family, TREM1 and TREM2, display ~20% sequence homology as well as some homology with other members of the Ig-SF such as activating NK cells receptors (20% identity with NKp44) and act through association with a DAP12- mediated pathway for signaling.

[0003] TREM2 was originally cloned as a cDNA encoding a TREM1 homologue (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22). This receptor is a glycoprotein of about 40kDa, which is reduced to 26kDa after N-deglycosylation. The TREM2 gene encodes a 230 amino acid-length protein that includes an extracellular domain, a transmembrane region and a short cytoplasmic tail. The extracellular region, encoded by exon 2, is composed of a single type V Ig-SF domain, containing three potential N-glycosylation sites. The putative transmembrane region
contains a charged lysine residue. The cytoplasmic tail of TREM2 lacks signaling motifs and is thought to signal through the signaling adaptor molecule DAP12/TRYROBP.

[0004] The signaling adaptor molecule DAP12 is expressed as a homodimer at the surface of a variety of cells participating in innate immune response, including microglia, macrophages, granulocytes, NK cells, and dendritic cells (DC). DAP12 is a member of the type I transmembrane adapter protein family on the basis of homology with the human T-cell receptor (TCR)-associated CD3 chains and the Fc receptor (FcR) γ-chain (Turnbull, IR and Colonna, M, Nat Rev Immunol, 2007. 7(2): p. 155-61). These proteins share many structural and functional characteristics, including one or more ITAM motifs in their cytoplasmic domain, charged acidic residue in transmembrane region (critical for interaction with its partner chain) and the ability to recruit Src homology domain-2 (SH2)-containing proteins following tyrosine phosphorylation. The ITAM motif mediates signal propagation by activation of the ZAP70 or Syk tyrosine kinase. Both kinases phosphorylate several substrates, thereby facilitating the formation of a signaling complex leading to cellular activation. Interestingly, some B-cells and T-cells also express DAP12 under inflammatory conditions. In humans, subsets of CD4⁺CD28⁻ T-cells, αβTCR⁺CD4⁺ T-cells, and CD8⁺ T-cells expressing this protein have been described in patients suffering from chronic inflammatory diseases, in the context of autoimmune T cells (Schleinitz, N. et al., PLoS ONE, 4 (2009), p. e6264). In view of the significant level of DAP12 expression in mouse peritoneal macrophages, this protein is believed to be expressed in other macrophage-related cells, such as osteoclasts in the bone marrow, Kupffer cells in the liver, alveolar macrophages of the lung, Langerhans cells of skin, and microglial cells in the brain (Takaki, R et al., Immunol Rev, 2006. 214: p. 118-29).

[0005] TREM2 has been identified as expressed on the surface of human monocyte-derived dendritic cells and as an mRNA transcript in the mouse macrophage cell line RAW264 (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22). Human TREM2 was the first DAP12-associated receptor described on the surface of DCs. Studies have demonstrated that TREM2 cell surface expression is reduced in DAP12-deficient bone marrow-derived dendritic cells (BMDCs) and in DAP12-deficient macrophages, as compared to wild-type cells (Ito, H and Hamerman, JA, Eur J Immunol. 42(1): p. 176-85; Hamerman, JA et al., J Immunol, 2006. 177(4): p. 2051-5; and

Recent studies have also shown cell-surface expression of TREM2 on macrophages infiltrating tissue from the circulation, as well as on macrophages activated by IL-4 or IL-13 (Tumbull, IR et al., J Immunol, 2006. 177(6): p. 3520-4). However, TREM2 expression was not always found in other cell populations, such as tissue-resident macrophages, circulating monocytes, or the corresponding progenitor cells in the bone marrow, suggesting that TREM2 expression is not induced centrally, but locally during tissue infiltration or by cytokine-mediated activation. Moreover, it has also been observed that IFN-γ and LPS reduce TREM2 expression. Further, it has been recently reported that TREM2 is highly expressed on microglia and infiltrating macrophages in the central nervous system during experimental autoimmune encephalomyelitis or Alzheimer’s disease (Piccio, L et al., Eur J Immunol, 2007. 37(5): p. 1290-301; and Wang Y, Cell. 2015 Mar 12;160(6):1061-71).

It has been shown that TREM2 signals through DAP12. Downstream this leads to activation of the Syk/Zap70 tyrosine kinase family, PI3K, and other intracellular signals. On myeloid cells, TLR signals are important for activation, such as with infection response, but also play a key role in the pathological inflammatory response, such as with macrophages and dendritic cells (Hamerman, JA et al., (2006) J Immunol 177: 2051-2055; Ito, H et al., Eur J Immunol 42: 176-185; Neumann, H et al., (2007) J Neuroimmunol 184: 92-99; Takahashi, K et al., (2005) J Exp Med 201: 647-657; and Takahashi, K et al., (2007) PLoS Med 4: e124). Deficiency of either TREM2 or DAP12 is thought to lead to increased pro-inflammatory signaling. The impact of TREM2-deficiency in vitro has been shown in the context of stimulation with typical TLR ligands, such as LPS, CpG DNA, and Zymosan. TREM-2-deficient dendritic cells show increased release of IL-12p70, TNF, IL-6, and IL-10 in the presence, but not in the absence of stimulation.

Several recent studies have explored the intracellular signaling events induced by the activation of the TREM2/DAP12 pathway. For example, TREM2 is thought to activate signaling pathways involved in cell survival (e.g., protein kinase B-Akt), cell activation and differentiation
(e.g., Syk, Erk1/2, PLC-γ, etc.), and in the control of the actin cytoskeleton (e.g., Syk, Vav, etc.) (Peng, Q et al., Sci Signal. 3(122): p. ra38; and Whittaker, GC et al., J Biol Chem. 285(5): p. 2976-85). After ligation of TREM2, the ITAM tyrosines in DAP12 are phosphorylated by SRC-family kinases leading to the recruitment and activation of the Syk kinase and/or ZAP70 kinase. In the mouse, Syk may be the predominant kinase involved, whereas in humans both Syk and ZAP70 appear to couple efficiently with such ITAM-containing subunits, binding them through their tandem SH2 domains.

Studies on TREM2 signaling have shown that, like TREM1, TREM2-mediated signaling through DAP12 also leads to an increase in intracellular calcium ion levels and ERK1/2 phosphorylation of ERK1/2 (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22; and Sharif, O and Knapp, S, Immunobiology. 2008. 213(9-10): p. 701-13). Importantly, TREM2 receptor ligation may not induce the degradation of IκB-α and the subsequent nuclear translocation of NF-kB, which points to a possible difference between TREM2 and TREM1 signaling (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22). Receptor cross-linking of TREM2 on immature dendritic cells triggers the up-regulation of molecules involved in T-cell co-stimulation, such as CD86, CD40, and MHC class II, as well as the up-regulation of the chemokine receptor CCR7 (Bouchon, A et al., J Exp Med, 2001. 194(8): p. 1111-22). TREM2 is also expressed on microglia, where receptor cross-linking results in an increase in ERK1/2 phosphorylation and CCR7, but not an increase in CD86 or MHC class II expression, suggesting possible cell type-specific differences in TREM2 signaling. Additionally, over expression of TREM2 signaling in microglia, myeloid Precursors, CHO or EK293 cells results in an increase in phagocytosis of apoptotic neurons, nerve and non-nerve tissue debris in the nervous system, disease causing proteins, bacteria and other foreign invaders, which is accompanied by a polarization and re-organization of F-actin in an ERK-dependent manner (Takahashi, K et al., PLoS Med, 2007. 4(4): p. e124; Neumann, H and Takahashi, K, J Neuroimmunol, 2007. 184(1-2): p. 92-9; and Kleinberg et al., Sci Transl Med. 2014 Jul 2;6(243):243ra86). However in some physiological contexts such as Pneumococcal Pneumonia, Trem2 appear to decrease phagocytosis. Thus TREM 2 deficient alveolar macrophages display augmented bacterial clearance from the lung

[0010] It has also been shown that bone marrow-derived macrophages (BMDM) that have been silenced for TREM2 using shRNAi display increased secretion of TNF in response to the TLR2/6 ligand zymosan and the TLR9 ligand CpG, as compared to control BMDM cells that were treated with a non-specific shRNAi, indicating that TREM2 negatively regulates cytokine synthesis in macrophages (Ito, H and Hamerman, JA, Eur J Immunol. 42(1): p. 176-85; Hamerman, JA et al., J Immunol, 2006. 177(4): p. 2051-5; and Hamerman, JA et al., Nat Immunol, 2005. 6(6): p. 579-86). These results have been confirmed using BMDM cells from TREM2 knockout mice, and have further shown that levels of TNF and IL-6 were also higher in TREM2\(-/-\) BMDM cells in response to LPS, as compared to wild-type BMDM cells (Turnbull, IR, et al., J Immunol, 2006. 177(6): p. 3520-4; and Turnbull, IR and Colonna, M, Nat Rev Immunol, 2007. 7(2): p. 155-61). Additionally, TREM2 overexpression in microglia has been demonstrated to lead to a decrease in TNF and inducible nitric oxide (iNOS) mRNA after culture of these cells with apoptotic neurons, whereas TREM2 knockdown resulted in a modest increase in TNF and iNOS mRNA levels. This indicates that, in contrast to TREM1, which is a positive regulator of cytokine synthesis, TREM2 is a negative regulator of cytokine synthesis. This effect of TREM2 on inflammation was thought to be independent of the type of macrophage as it occurs in both microglia and BMDM cells.

[0011] It has also been shown that in resident myeloid cells of the central nervous system, activation of microglia can lead to inflammation (Neumann, H et al., (2007) J Neuroimmunol 184: 92-99; Takahashi, K et al., (2005) J Exp Med 201: 647-657; Takahashi, K et al., (2007) PLoS Med 4: e124; and Hsieh, CL et al., (2009) J Neurochem 109: 1144-1156). Moreover, microglia activation has also been implicated in frontotemporal dementia (FTD), Alzheimer’s disease, Parkinson’s disease, stroke/ischemic brain injury, and multiple sclerosis. Whereas reduced TREM2 activation leads to increases in certain activation and inflammation markers, such as NOS2 gene transcription in myeloid cells, increased TREM2 activation leads to reduced NOS2 transcription. It is thought that dying neurons express an endogenous ligand for TREM2. HSP60 has been implicated as a ligand of TREM2 on neuroblastoma cells (Stefani, L et al.,
(2009) Neurochem 110: 284-294). TREM2 over-expression also leads to increased phagocytosis of dying neurons by microglia, and similarly increases phagocytosis by other myeloid lineage cells. Trem2 has also been implicated in myeloid cell migration, as Trem2 deficient myeloid cells fail to populate the brain of rodent models for Alzheimer’s disease (Malm, TM et al, Neurotherapeutics. 2014 Nov 18).


[0013] TREM2 gene expression has also been shown to be increased in APP23 transgenic mice, an Alzheimer’s disease model in which the mice express a mutant form of the amyloid precursor protein that is associated with familial Alzheimer’s disease (Melchior, B et al., ASN Neuro 2: e00037). Uptake of Amyloid 1-42 has also been shown to be increased in BV-2 microglial cell lines that overexpress TREM2.


[0015] Further, exome sequencing of individuals with frontotemporal dementia (FTD) presentation has identified homozygous mutations in TREM2 (Guerreiro, RJ et al., JAMA Neurol 70: 78-84; and Guerreiro, RJ et al., Arch Neurol: 1-7). Some of these mutations lead to truncation and likely loss-of-function of TREM2. While others involve changes in amino acids including, Q33X, R47H, T66M, and S116C (Borroni B, et al. Neurobiol Aging. 2014 Apr;35(4):934.e7-10). Imaging analysis in certain individuals with TREM2 homozygous mutations has also shown evidence of demyelination.
Heterozygous mutations in TREM2, which are the same as the mutation that cause Naku-Hakula and FTD, as well as TREM2 variants, also increase the risk of Alzheimer’s disease (Guerreiro, R et al., N Engl J Med 368: 117-127; Jonsson, T et al., N Engl J Med 368: 107-116; and Neumann, H et al., N Engl J Med 368: 182-184). Although these TREM2 mutations are rarer than the known risk variants of Alzheimer’s disease (e.g., APOE4), the effect of carrying these mutations is just as serious; around a 3 fold increase in the risk of developing Alzheimer’s disease. Moreover, even individuals without Alzheimer’s disease who carry a heterozygous TREM2 mutation show worse cognition as compared to individuals with two normal TREM2 alleles. Further, it has been shown that the R47H variant of TREM2 (arginine to histidine amino acid substitution at position 47 of TREM2), which is most common TREM2 mutation is located within the immunoglobulin domain of TREM2, and may thus alter ligand binding (Wang Y, Cell. 2015 Mar 12;160(6):1061-71). TREM2 was also shown to be required for survival of microglia in the brain (Otero et al., (2009) Nat Immunol.;10:734-43). In summary, Trem2 variants were identified as genetic risk factors for frontotemporal dementia, Parkinson’s disease, and amyotrophic lateral sclerosis (Borroni B, et al. Neurobiol Aging. 2014 Apr;35(4):934.e7-10; Rayaprolu S, et al., Mol Neurodegener. 2013 Jun 21:8:19; and Cady J, et al., JAMA Neurol. 2014 Apr;71(4):449-53). This common genetic linkage suggests a more general role for Trem2 in modulating neurodegenerative disease pathology.

In addition an integrative network-based approach to rank-ordered organized structure of molecular networks of gene expression for relevance to late onset developing Alzheimer’s disease (LOAD) identified TYROBP/DAP12 as the signaling molecule for TREM2 as a key regulator of the immune/microglia gene modules that is associated with LOAD. TYROBP was found to be the causal regulator of the highest scoring immune/ microglia module as rank-ordered based on the number of other genes that TREM2 regulated and the magnitude of loss of regulation, as well as differential expression in LOAD brains. TYROBP was significantly upregulated in LOAD brains and there was a progression of TYROBP expression changes across mild cognitive impairment (MCI), which often precedes LOAD (Zhang et al., (2013) Cell 153, 707-720; and Ma et al., Mol Neurobiol. 2014 Jul 23). Targeting such causal networks in ways that restore them to a normal state may be a way to treat disease.
Accordingly, there is a need for antibodies that specifically bind TREM2 on a cell surface and that modulate (e.g., activate) one or more TREM2 activities in order to treat one or more diseases, disorders, and conditions associated with decreased TREM2 activity.

Moreover, the tumor microenvironment is composed of a heterogeneous immune infiltrate, which include T lymphocytes, macrophages and cells of myeloid/granulocytic lineage. Therapeutic approaches that modulate specific subsets of immune cells are changing the standard of care. “Checkpoint blocking” antibodies targeting immune-modulatory molecules expressed on T cells (such as CTLA-4 and PD-1) have demonstrated clinical activity across a variety of tumor types (Naidoo et al., 2014 *British Journal of Cancer* 111, 2214–2219).

Cancer immune-therapy targeting tumor-associated macrophages (e.g., M2-type macrophages) is an intense area of research. The presence of M2-macrophages in tumors is associated with poor prognosis.

Accordingly, there is a need for antibodies that specifically bind TREM2 on a cell surface and modulate (e.g., inhibit and/or otherwise reduce) ligand binding and/or one or more TREM2 activities in order to prevent, reduce the risk of, or treat cancer.

All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

**SUMMARY OF THE INVENTION**

The invention is generally directed to methods and compositions that include antibodies, e.g., monoclonal antibodies, chimeric antibodies, bispecific antibodies, humanized antibodies, antibody fragments, etc., that specifically bind a TREM2 protein e.g., a mammalian TREM2, a human TREM2, including wild-type proteins and naturally occurring variants thereof. The antibodies of the present disclosure may include agonist, antagonist, or inert antibodies. The methods provided herein find use in preventing, reducing risk, or treating an individual having dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, Huntington’s disease, amyotrophic lateral sclerosis, Taupathy disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma,
lupus, acute and chronic colitis, wound healing, Crohn's disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet's disease, Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear palsy, cortical basal ganglionic degeneration, acute disseminated encephalomyelitis, granulomatous disorders, Sarcoidosis, diseases of aging, seizures, spinal cord injury, traumatic brain injury, age related macular degeneration, glaucoma, retinitis pigmentosa, retinal degeneration, respiratory tract infection, eye infection, systemic infection, lupus, arthritis, multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrotic disease, Paget's disease of bone. In some embodiments, tumor cells, such as acute myeloblastic leukemia (AML) cell, express TREM2. Accordingly, anti-TREM2 antibodies of the present disclosure also find use in treating cancers. In some embodiments, anti-TREM2 antibodies, including antibodies that display antibody-dependent cell-mediated cytotoxicity (ADCC) and/or TREM2 antibody drug conjugates, can be used to target and inhibit cancer, such as AML.

[0024] Certain aspects of the present disclosure relate to an isolated agonist antibody that binds to a TREM2 protein, wherein the antibody induces one or more TREM2 activities.

[0025] In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein is a mammalian protein or a human protein. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein is a wild-type protein. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein is a naturally occurring variant. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein is expressed on human dendritic cells, human macrophages, human monocytes, human osteoclasts, human Langerhans cells of skin, human Kupffer cells, and/or human microglia. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody induces or retains TREM2 clustering on a cell surface. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise TREM2 binding to DAP12. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise TREM2 autophosphorylation. In
certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise DAP12 phosphorylation. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 autophosphorylation, the DAP12 phosphorylation, or both is induced by one or more SRC family tyrosine kinases. In certain embodiments that may be combined with any of the preceding embodiments, the one or more SRC family tyrosine kinases comprise a Syk kinase. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise PI3K activation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise increased expression of one or more anti-inflammatory cytokines. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise increased expression of one or more cytokines selected from the group consisting of IL-12p70, IL-6, and IL-10. In certain embodiments that may be combined with any of the preceding embodiments, the increased expression occurs in one or more cells selected from the group consisting of macrophages, dendritic cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and microglial cells. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise reduced expression of one or more pro-inflammatory cytokines. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise reduced expression of one or more pro-inflammatory mediators selected from the group consisting of IFN-α4, IFN-β, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, and CRP. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise reduced expression of TNF-α, IL-6, or both. In certain embodiments that may be combined with any of the preceding embodiments, the reduced expression of the one or more pro-inflammatory mediators occurs in one or more cells selected from the group consisting of macrophages, dendritic cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and microglial cells. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise
extracellular signal-regulated kinase (ERK) phosphorylation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise increased expression of C-C chemokine receptor 7 (CCR7). In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise an increased ability of dendritic cells, monocytes, microglia, and/or macrophages to induce T-cell proliferation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise an enhancement, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise induction of osteoclast production, increased rate of osteoclastogenesis, or both. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise increasing the survival of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise increasing the function of dendritic cells, macrophages, and/or microglia. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise increasing phagocytosis by dendritic cells, macrophages, monocytes, and/or microglia under conditions of reduced levels of M-CSF. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise decreasing phagocytosis by dendritic cells, macrophages, monocytes, and/or microglia in the presence of normal levels of M-CSF. In certain embodiments that may be combined with any of the preceding embodiments, the macrophages and/or microglia are M1 macrophages and/or microglia, M2 macrophages and/or microglia, or both. In certain embodiments that may be combined with any of the preceding embodiments, the M1 macrophages and/or microglia are activated M1 macrophages and/or microglia. In certain embodiments that may be combined with any of the preceding
embodiments, the one or more TREM2 activities comprise induction of one or more types of clearance selected from the group consisting of apoptotic neuron clearance, nerve tissue debris clearance, non-nerve tissue debris clearance, bacteria or other foreign body clearance, disease-causing protein clearance, and tumor cell clearance. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise induction of phagocytosis of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, disease-causing proteins, disease-causing peptides, disease-causing nucleic acid, or tumor cells. In certain embodiments that may be combined with any of the preceding embodiments, the disease-causing nucleic acid is antisense GGCCCC (G2C4) repeat-expansion RNA. In certain embodiments that may be combined with any of the preceding embodiments, the disease-causing protein is selected from the group consisting of amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein AI, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, and proline-arginine (PR) repeat peptides. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise normalization of disrupted TREM2/DAP12-dependent gene expression. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise recruitment of Syk, ZAP70, or both to a DAP12/TREM2 complex. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise Syk phosphorylation. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise increased expression of CD83 and/or CD86 on dendritic cells, macrophages, and/or monocytes. In certain embodiments that may be combined with any of the preceding embodiments, the dendritic cells are bone marrow-derived dendritic cells. In certain embodiments that may be combined with any of the
preceding embodiments, the one or more TREM2 activities comprise reduced secretion of one or more inflammatory cytokines. In certain embodiments that may be combined with any of the preceding embodiments, the one or more inflammatory cytokines are selected from the group consisting of IFN-α4, IFN-β, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, CRP, and MCP-1. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise reduced expression of one or more inflammatory receptors. In certain embodiments that may be combined with any of the preceding embodiments, the one or more inflammatory receptors comprise CD86. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise increasing activity of one or more TREM2-dependent genes. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2-dependent genes comprise one or more nuclear factor of activated T-cells (NFAT) transcription factors. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is of the IgG class the IgM class, or the IgA class. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is of the IgG class and has an IgG1, IgG2, IgG3, or IgG4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG2 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG2 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG2 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody induces the one or more TREM2 activities independently of binding to an Fc receptor. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds an inhibitory Fc receptor. In certain embodiments that may be combined with any of the preceding embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγIIIB). In certain embodiments that may be combined with any of the preceding embodiments, the human IgG2 constant region comprises an Fc region that comprises one or more modifications. In certain embodiments that may be
combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of V234A, G237A, H268Q, V309L, A330S, P331S, C232S, C233S, S267E, L328F, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the human \( \text{IgG2} \) constant region comprises a light chain constant region comprising a C214S amino acid substitution, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an \( \text{IgG1} \) isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human \( \text{IgG1} \) constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human \( \text{IgG1} \) constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds an inhibitory Fc receptor. In certain embodiments that may be combined with any of the preceding embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIb (\( \text{Fc} \gamma \text{RIIB} \)). In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of N297A, D265A, L234A, L235A, G237A, C226S, C229S, E233P, L234V, L234F, L235E, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises an \( \text{IgG2} \) isotype heavy chain constant domain 1(CH1) and hinge region. In certain embodiments that may be combined with any of the preceding embodiments, the \( \text{IgG2} \) isotype CH1 and hinge region comprise the amino acid sequence of ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS
WNSGALTSGVHTPAPLQSS GLYSLSVVT VPSSNFGTQT YTCNVDDHKPS NTKVDKTVKCCVECPFC (SEQ ID NO: 397). In certain embodiments that may be combined with any of the preceding embodiments, the antibody Fc region comprises a S267E amino acid substitution, a L328F amino acid substitution, or both, and/or a N297A or N297Q amino acid substitution, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a mouse IgG1 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG4 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG4 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds an inhibitory Fc receptor. In certain embodiments that may be combined with any of the preceding embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcyIIIB). In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of L235A, G237A, S228P, L236E, S267E, E318A, L328F, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has a hybrid IgG2/4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises an amino acid sequence comprising amino acids 118 to 260 of human IgG2 and amino acids 261 to 447 of human IgG4, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a mouse IgG4 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is an antibody fragment that binds to
one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of human TREM2, and wherein the antibody fragment is cross-linked to a second antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of human TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the fragment is an Fab, Fab’, Fab’-SH, F(ab’)2, Fv or scFv fragment.

[0026] Other aspects of the present disclosure relate to an isolated inert antibody that binds to a TREM2 protein. Other aspects of the present disclosure relate to an isolated antagonist antibody that binds to a TREM2 protein.

[0027] In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein is a mammalian protein or a human protein. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein is a wild-type protein. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein is a naturally occurring variant. In certain embodiments that may be combined with any of the preceding embodiments, the TREM2 protein is a disease variant. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody inhibits one or more TREM2 activities. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise decreasing activity of one or more TREM2-dependent genes. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2-dependent genes comprise one or more nuclear factor of activated T-cells (NFAT) transcription factors. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities comprise decreasing the survival of macrophages, microglial cells, M1 macrophages, M1 microglial cells, M2 macrophages, M2 microglial cells, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or dendritic cells. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody inhibits interaction between TREM2 and one or more TREM2 ligands, inhibits TREM2 signal transduction, or both. In certain embodiments that may be combined with any of the preceding embodiments, the
antibody is incapable of binding an Fc-gamma receptor (FcγR). In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG1 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG1 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG1 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of N297A, N297Q, D265A, L234A, L235A, C226S, C229S, P238S, E233P, L234V, P238A, A327Q, A327G, P329A, K322A, L234F, L235E, P331S, T394D, A330L, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region further comprises an amino acid deletion at a position corresponding to glycine 236 according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a mouse IgG1 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG2 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG2 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG2 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of V234A, G237A, H268E, V309L, N297A, N297Q, A330S, P331S, C232S, C233S, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is
according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG4 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG4 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of E233P, F234V, L235A, G237A, E318A, S228P, L236E, S241P, L248E, T394D, M252Y, S254T, T256E, N297A, N297Q, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is an antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the fragment is an Fab, Fab’, Fab’-SH, F(ab’)_2, Fv or scFv fragment.

[0028] In certain embodiments that may be combined with any of the preceding embodiments, the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of A330L, L234F, L235E, P331S, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region further comprises a S228P amino acid substitution according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody competes for binding of TREM2
with one or more TREM2 ligands. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 ligands are selected from the group consisting of E. coli cells, apoptotic cells, nucleic acids, anionic lipids, zwitterionic lipids, negatively charged phospholipids, phosphatidylserine, sulfatides, phosphatidylcholin, sphingomyelin, membrane phospholipids, lipated proteins, proteolipids, lipated peptides, and lipated amyloid beta peptide. In certain embodiments that may be combined with any of the preceding embodiments, the antibody does not compete for binding of TREM2 with a TREM2 ligand. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a human antibody, a humanized antibody, a bispecific antibody, a multivalent antibody, a conjugated antibody, or a chimeric antibody. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a bispecific antibody recognizing a first antigen and a second antigen. In certain embodiments that may be combined with any of the preceding embodiments, the first antigen is human TREM2 or a naturally occurring variant thereof, and the second antigen is a disease-causing protein selected from the group consisting of amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein AI, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, and proline-arginine (PR) repeat peptides; a blood brain barrier targeting protein selected from the group consisting of: transreceptor, insulin receptor, insulin like growth factor receptor, LRP-1, and LRP1; or ligands and/or proteins expressed on immune cells, wherein the ligands and/or proteins selected from the group consisting of: CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, BTLA, KIR, GAL9, TIM3, A2AR, LAG, and phosphatidylycerine. In certain embodiments that may be combined with any of the preceding embodiments, the first antigen is human TREM2 or a naturally occurring variant thereof, and the second antigen is a protein expressed on one or more tumor cells. In
certain embodiments that may be combined with any of the preceding embodiments, the antibody is an antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, human DAP12, and naturally occurring variant of human DAP12; and wherein the antibody is used in combination with one or more antibodies that specifically bind a disease-causing protein selected from the group consisting of: amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein A1, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, and proline-arginine (PR) repeat peptides, and any combination thereof, or with one or more antibodies that specifically bind a cancer-associated protein selected from the group consisting of: CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, BTLA, KIR, GAL9, TIM3, A2AR, LAG, phosphatidylinerine, and any combination thereof. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a monoclonal antibody.

[0029] In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody binds to a linear epitope on TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the linear epitope on TREM2 is located within the extracellular domain of TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the linear epitope on TREM2 is located within the extracellular immunoglobulin-like variable-type (IgV) domain of TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the linear epitope on TREM2 comprises one or more amino acid residues located within the extracellular immunoglobulin-like variable-type (IgV) domain of TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody binds to a conformational epitope on TREM2. In certain embodiments that may be combined with any of
the preceding embodiments, the conformational epitope on TREM2 comprises one or more amino acid residues located within the extracellular domain of TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the conformational epitope on TREM2 comprises one or more amino acid residues located within the extracellular immunoglobulin-like variable-type (IgV) domain of TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds to one or more amino acids within amino acid residues selected from the group consisting of: i. amino acid residues 130-171 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 130-171 of SEQ ID NO: 1; ii. amino acid residues 139-153 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 139-153 of SEQ ID NO: 1; iii. amino acid residues 139-146 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 139-146 of SEQ ID NO: 1; iv. amino acid residues 130-144 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 130-144 of SEQ ID NO: 1; and v. amino acid residues 158-171 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 158-171 of SEQ ID NO: 1. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds to an epitope comprising one or more amino acids within amino acid residues selected from the group consisting of: i. amino acid residues 130-171 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 130-171 of SEQ ID NO: 1; ii. amino acid residues 139-153 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 139-153 of SEQ ID NO: 1; iii. amino acid residues 139-146 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 139-146 of SEQ ID NO: 1; iv. amino acid residues 130-144 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 130-144 of SEQ ID NO: 1; and v. amino acid residues 158-171 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 158-171 of SEQ ID NO: 1. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds to an epitope further comprising one or more amino acid residues selected from the group consisting of: i. amino acid residue Arg47 or Asp87 of SEQ ID
NO: 1; ii. amino acid residues 40-44 of SEQ ID NO: 1; iii. amino acid residues 67-76 of SEQ ID NO: 1; and iv. amino acid residues 114-118 of SEQ ID NO: 1.

[0030] In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an HVR-H1, HVR-H2, and/or HVR-H3 of a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87, and/or wherein the light chain variable domain comprises an HVR-L1, HVR-L2, and/or HVR-L3 of a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-L2
comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises: (a) an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24; (b) a an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49; and (c) an HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 50-119; and/or wherein the light chain variable domain comprises (a) a an HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137; (b) an HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152; and (c) an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236 or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152.

[0031] Other aspects of the present disclosure relate to an isolated anti-human TREM2 antibody, wherein the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and/or HVR-H3 of a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32,
Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87, and/or wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and/or HVR-L3 of a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-L2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152. In certain embodiments that may be combined with any of the preceding embodiments, the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24, an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49, and an HVR-H3
comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119, and/or wherein the light chain variable domain comprises an HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137, an HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152, and an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236.

[0032] Other aspects of the present disclosure relate to an isolated anti-human TREM2 antibody which binds essentially the same TREM2 epitope as a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab52, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87.

[0033] Other aspects of the present disclosure relate to an isolated anti-human TREM2 antibody, wherein the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises: (a) an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24; (b) an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49; and (c) an HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119; and/or wherein the light chain variable domain comprises (a) a HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the
group consisting of SEQ ID NOs:120-137; (b) an HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152; and (c) an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236 or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152.

[0034] In certain embodiments that may be combined with any of the preceding embodiments, the antibody is an agonist antibody, and wherein the antibody induces one or more TREM2 activities. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody induces or retains TREM2 clustering on a cell surface. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities are selected from the group consisting of TREM2 binding to DAP12; DAP12 binding to TREM2; TREM2 phosphorylation, DAP12 phosphorylation; PI3K activation; increased expression of one or more cytokines selected from the group consisting of IL-12p70, IL-6, and IL-10; reduced expression of one or more pro-inflammatory mediators selected from the group consisting of IFN-a4, IFN-b, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, and CRP; reduced expression of TNF-α, IL-6, or both; extracellular signal-regulated kinase (ERK) phosphorylation; increased expression of C-C chemokine receptor 7 (CCR7); induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells; increased ability of dendritic cells, monocytes, microglia, and/or macrophages to induce T-cell proliferation; an increase, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation; induction of osteoclast production, increased rate of osteoclastogenesis, or both; increasing the survival and/or function of one or more of dendritic cells, macrophages, M1 macrophages, activated M1 macrophages M2 macrophages, osteoclasts, Langerhans cells of skin, Kupffer cells, microglial cells, M1 microglial cells, activated M1 microglial cells, and M2 microglial cells; induction of one or more types of clearance selected from the group consisting of cancer cells
clearance, apoptotic neuron clearance, nerve tissue debris clearance, non-nerve tissue debris clearance, bacteria or other foreign body clearance, and disease-causing protein clearance; induction of phagocytosis of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, disease-causing proteins or tumor cells; normalization of disrupted TREM2/DAP12-dependent gene expression; recruitment of Syk, ZAP70, or both to the TREM2/DAP12 complex; Syk phosphorylation; increased expression of CD83 and/or CD86 on dendritic cells, microglia, monocytes, or macrophages; reduced secretion of one or more inflammatory cytokines selected from the group consisting of IFN-a4, IFN-b, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, CRP, and MCP-1; reduced expression of one or more inflammatory receptors; increasing phagocytosis by macrophages, monocytes, dendritic cells, and/or microglia under conditions of reduced levels of MCSF; decreasing phagocytosis by macrophages, monocytes, dendritic cells, and microglia in the presence of normal levels of MCSF; increasing activity of one or more TREM2-dependent genes; and any combination thereof. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is of the IgG class the IgM class, or the IgA class. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is of the IgG class and has an IgG1, IgG2, IgG3, or IgG4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG2 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG2 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG2 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody induces the one or more TREM2 activities independently of binding to an Fc receptor. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds an inhibitory Fc receptor. In certain embodiments that may be combined with any of the preceding embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγIIIB). In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more
modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of V234A, G237A, H268Q, V309L, A330S, P331S, C232S, C233S, S267E, L328F, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG2 constant region comprises a light chain constant region comprising a C214S amino acid substitution, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG1 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG1 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG1 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds an inhibitory Fc receptor. In certain embodiments that may be combined with any of the preceding embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγRIIB). In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of N297A, D265A, L234A, L235A, G237A, C226S, C229S, E233P, L234V, L234F, L235E, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises an IgG2 isotype heavy chain constant domain 1(CH1) and hinge region. In certain embodiments that may be combined with any of the preceding embodiments, the IgG2 isotype CH1 and hinge region comprise the amino acid sequence of ASTKGPSVFP LAPCSRSTSE STAALGCLVK
DYFPEPVTVS WNSGALTSGVHTFPAPLQSS GLYSLSSVVT VPSSNFGTQT
YTCNVDHKPS NTKVDKTVERKCCVECPPCP (SEQ ID NO:397). In certain embodiments that may be combined with any of the preceding embodiments, the antibody Fc region comprises a S267E amino acid substitution, a L328F amino acid substitution, or both, and/or a N297A or N297Q amino acid substitution, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a mouse IgG1 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG4 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG4 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody binds an inhibitory Fc receptor. In certain embodiments that may be combined with any of the preceding embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγIIB). In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises amino acid substitutions in the Fc region are at a residue position selected from the group consisting of L235A, G237A, S228P, L236E, S267E, E318A, L328F, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has a hybrid IgG2/4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises an amino acid sequence comprising amino acids 118 to 260 of human IgG2 and amino acids 261 to 447 of human IgG4, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a mouse IgG4 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is an
antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of TREM2, and wherein the antibody fragment is cross-linked to a second antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the fragment is an Fab, Fab', Fab'-SH, F(ab')2, Fv or scFv fragment. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is an inert antibody. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is an antagonist antibody. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody inhibits one or more TREM2 activities. In certain embodiments that may be combined with any of the preceding embodiments, the one or more TREM2 activities are selected from the group consisting of decreasing activity of one or more TREM2-dependent genes; decreasing activity of one or more nuclear factor of activated T-cells (NFAT) transcription factors; decreasing the survival of macrophages, microglial cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or dendritic cells; and any combination thereof. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody inhibits interaction between TREM2 and one or more TREM2 ligands, inhibits TREM2 signal transduction, or both. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is incapable of binding an Fc-gamma receptor (FcγR). In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG1 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG1 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG1 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more
amino acid substitutions in the Fc region are at a residue position selected from the group consisting of N297A, N297Q, D265A, L234A, L235A, C226S, C229S, P238S, E233P, L234V, P238A, A327Q, A327G, P329A, K322A, L234F, L235E, P331S, T394D, A330L, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region further comprises an amino acid deletion at a position corresponding to glycine 236 according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a mouse IgG1 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG2 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG2 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG2 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of V234A, G237A, H268E, V309L, N297A, N297Q, A330S, P331S, C232S, C233S, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG4 constant region. In certain embodiments that may be combined with any of the preceding embodiments, the human IgG4 constant region comprises an Fc region. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more modifications. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region comprises one or more amino acid substitutions. In certain embodiments that may be combined with any of the preceding embodiments, the one or more amino acid substitutions in the Fc region are at a
residue position selected from the group consisting of E233P, F234V, L235A, G237A, E318A, S228P, L236E, S241P, L248E, T394D, M252Y, S254T, T256E, N297A, N297Q, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is an antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the fragment is an Fab, Fab', Fab'-SH, F(ab')2, Fv or scFv fragment. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of A330L, L234F, L235E, P331S, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the Fc region further comprises a serine to proline amino acid substitution at position 228 according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a human antibody, a humanized antibody, a bispecific antibody, a multivalent antibody, or a chimeric antibody. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a bispecific antibody recognizing a first antigen and a second antigen. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a monoclonal antibody.

[0035] In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody binds specifically to both human TREM2 and mouse TREM2. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody has dissociation constant (K_D) for human TREM2 and mouse TREM2 that ranges from less than about 6.70 nM to less than about 0.23 nM. In certain embodiments that
may be combined with any of the preceding embodiments, the isolated antibody has dissociation constant (K_D) for human TREM2-Fc fusion protein that ranges from less than about 0.71 nM to less than about 0.23 nM. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody has dissociation constant (K_D) for human monomeric TREM2 protein that ranges from less than about 6.70 nM to less than about 0.66 nM. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody has dissociation constant (K_D) for mouse TREM2-Fc fusion protein that ranges from less than about 4.90 nM to less than about 0.35 nM.

[0036] Other aspects of the present disclosure relate to an isolated nucleic acid encoding the antibody of any one of the preceding embodiments. Other aspects of the present disclosure relate to a vector comprising the nucleic acid of any one of the preceding embodiments. Other aspects of the present disclosure relate to a host cell comprising the vector of any one of the preceding embodiments. Other aspects of the present disclosure relate to a method of producing an antibody, comprising culturing the cell of any one of the preceding embodiments so that the antibody is produced. In certain embodiments, the method further comprises recovering the antibody produced by the cell. Other aspects of the present disclosure relate to a pharmaceutical composition comprising the antibody of any one of the preceding embodiments and a pharmaceutically acceptable carrier.

[0037] Other aspects of the present disclosure relate to a method of preventing, reducing risk, or treating an individual having a disease, disorder, or injury selected from the group consisting of dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma, lupus, acute and chronic colitis, wound healing, Crohn’s disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet’s disease, Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear palsy, cortical basal ganglionic degeneration, acute disseminated encephalomyelitis, granulomatous disorders, Sarcoidosis, diseases of aging, seizures, spinal cord injury, traumatic brain injury, age related macular degeneration, glaucoma,
retinitis pigmentosa, retinal degeneration, respiratory tract infection, sepsis, eye infection, systemic infection, lupus, arthritis, multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrosic disease, Paget's disease of bone, and cancer. comprising administering to the individual a therapeutically effective amount of an isolated antibody of any one of the preceding embodiments. Other aspects of the present disclosure relate to an isolated antibody of any one of the preceding embodiments for use in preventing, reducing risk, or treating an individual having a disease, disorder, or injury selected from the group consisting of dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma, lupus, acute and chronic colitis, wound healing, Crohn's disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet’s disease, Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear palsy, cortical basal ganglionic degeneration, acute disseminated encephalomyelitis, granulomatous disorders, Sarcoidosis, diseases of aging, seizures, spinal cord injury, traumatic brain injury, age related macular degeneration, glaucoma, retinitis pigmentosa, retinal degeneration, respiratory tract infection, sepsis, eye infection, systemic infection, lupus, arthritis, multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrosic disease, Paget's disease of bone, and cancer. Other aspects of the present disclosure relate to use of an isolated antibody of any one of the preceding embodiments in the manufacture of a medicament for preventing, reducing risk, or treating an individual having a disease, disorder, or injury selected from the group consisting of dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma, lupus, acute and chronic colitis, wound healing, Crohn's disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet’s disease, Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear palsy, cortical basal ganglionic degeneration, acute disseminated
encephalomyelitis, granulomatous disorders, Sarcoidosis, diseases of aging, seizures, spinal cord injury, traumatic brain injury, age related macular degeneration, glaucoma, retinitis pigmentosa, retinal degeneration, respiratory tract infection, sepsis, eye infection, systemic infection, lupus, arthritis, multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrosic disease, Paget's disease of bone, and cancer.

[0038] Other aspects of the present disclosure relate to a method of inducing or promoting innate immune cell survival or wound healing an individual in need thereof, comprising administering to the individual a therapeutically effective amount of an isolated agonist antibody that binds to a TREM2 protein. Other aspects of the present disclosure relate to an isolated agonist antibody that binds to a TREM2 protein for use in inducing or promoting innate immune cell survival or wound healing an individual in need thereof. Other aspects of the present disclosure relate to use of an isolated agonist antibody that binds to a TREM2 protein in the manufacture of a medicament for inducing or promoting innate immune cell survival or wound healing an individual in need thereof.

[0039] In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is: (a) an agonist antibody; (b) an inert antibody; or an (c) an antagonist antibody. In certain embodiments that may be combined with any of the preceding embodiments, (a) the antibody is of the IgG class the IgM class, or the IgA class; and/or(b) the antibody has an IgG1, IgG2, IgG3, or IgG4 isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody comprises one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of: (a) V234A, G237A, H268Q, V309L, A330S, P331S, C232S, C233S, S267E, L328F, M252Y, S254T, T256E, and any combination thereof; (b) N297A, D265A, L234A, L235A, G237A, C226S, C229S, E233P, L234V, L234F, L235E, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, and any combination thereof; (c) L235A, G237A, S228P, L236E, S267E, E318A, L328F, M252Y, S254T, T256E, and any combination thereof; (d) N297A, N297Q, D265A, L234A, L235A, C226S, C229S, P238S, E233P, L234V, P238A, A327Q, A327G, P329A, K322A, L234F, L235E, P331S, T394D, A330L, M252Y, S254T, T256E, and any combination thereof; (e) V234A, G237A, H268E, V309L, N297A, N297Q, A330S, P331S, C232S, C233S, M252Y,
S254T, T256E, and any combination thereof; or (f) E233P, F234V, L235A, G237A, E318A, S228P, L236E, S241P, L248E, T394D, M252Y, S254T, T256E, N297A, N297Q, and any combination thereof, wherein the numbering of the residues is according to EU numbering. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody: (a) binds to one or more amino acids within amino acid residues 43-50 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 43-50 of SEQ ID NO: 1; or (b) one or more amino acids within amino acid residues 49-57 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 49-57 of SEQ ID NO: 1. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody: (a) binds essentially the same TREM2 epitope as the antibody Ab52; (b) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and/or HVR-H3 of the monoclonal antibody Ab52; and/or wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and/or HVR-L3 of the monoclonal antibody Ab52; (c) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:398, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:398, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:399, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:399, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:400, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:400, and/or wherein the light chain variable domain comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:401, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:401, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:402, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:402, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:403, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:403; (d) binds essentially the same TREM2 epitope as the antibody Ab21; (e) comprises a heavy chain variable domain and a light chain variable domain, wherein
the heavy chain variable domain comprises the HVR-H1, HVR-H2, and/or HVR-H3 of the monoclonal antibody Ab21; and/or wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and/or HVR-L3 of the monoclonal antibody Ab21; or (f) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:404, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:404, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:405, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:405, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:406, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:406, and/or wherein the light chain variable domain comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:407, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:407, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:408, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:408, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:409, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:409. In certain embodiments that may be combined with any of the preceding embodiments, the isolated antibody is the isolated antibody of any one of the preceding embodiments. In certain embodiments that may be combined with any of the preceding embodiments, the isolated agonist antibody is the isolated agonist antibody of any one of the preceding embodiments.

[0040] In certain embodiments that may be combined with any of the preceding embodiments, the individual has a heterozygous variant of TREM2, wherein the variant comprises one or more substitutions selected from the group consisting of: i. a glutamic acid to stop codon substitution in the nucleic acid sequence encoding amino acid residue Glu14 of SEQ ID NO: 1; ii. a glutamine to stop codon substitution in the nucleic acid sequence encoding amino acid residue Gln33 of SEQ ID NO: 1; iii. a tryptophan to stop codon substitution in the nucleic acid sequence encoding amino acid residue Trp44 of SEQ ID NO: 1; iv. an arginine to histidine amino acid substitution at an amino acid corresponding to amino acid residue Arg47 of SEQ ID
NO: 1; v. a tryptophan to stop codon substitution in the nucleic acid sequence encoding amino acid residue Trp78 of SEQ ID NO: 1; vi. a valine to glycine amino acid substitution at an amino acid corresponding to amino acid residue Val126 of SEQ ID NO: 1; vii. an aspartic acid to glycine amino acid substitution at an amino acid corresponding to amino acid residue Asp134 of SEQ ID NO: 1; and viii. a lysine to asparagine amino acid substitution at an amino acid corresponding to amino acid residue Lys186 of SEQ ID NO: 1. In certain embodiments that may be combined with any of the preceding embodiments, the individual has a heterozygous variant of TREM2, wherein the variant comprises a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G313 of the nucleic acid sequence encoding SEQ ID NO: 1; a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G267 of the nucleic acid sequence encoding SEQ ID NO: 1; or both. In certain embodiments that may be combined with any of the preceding embodiments, the individual has a heterozygous variant of DAP12, wherein the variant comprises one or more variants selected from the group consisting of: i. a methionine to threonine substitution at an amino acid corresponding to amino acid residue Met1 of SEQ ID NO: 2; ii. a glycine to arginine amino acid substitution at an amino acid corresponding to amino acid residue Gly49 of SEQ ID NO: 2; iii. a deletion within exons 1-4 of the nucleic acid sequence encoding SEQ ID NO: 2; iv. an insertion of 14 amino acid residues at exon 3 of the nucleic acid sequence encoding SEQ ID NO: 2; and v. a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G141 of the nucleic acid sequence encoding SEQ ID NO: 2.

[0041] In certain embodiments that may be combined with any of the preceding embodiments, the cancer is selected from the group consisting of bladder cancer, brain cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, kidney cancer, renal cell cancer, renal pelvis cancer, leukemia, lung cancer, melanoma, non-Hodgkin’s lymphoma, pancreatic cancer, prostate cancer, ovarian cancer, fibrosarcoma, and thyroid cancer. In certain embodiments that may be combined with any of the preceding embodiments, the method further comprises administering to the individual at least one antibody that specifically binds to an inhibitory checkpoint molecule, and/or another standard or investigational anti-cancer therapy. In certain embodiments that may be combined with any of the preceding embodiments, the at
least one antibody that specifically binds to an inhibitory checkpoint molecule is administered in combination with the isolated antibody. In certain embodiments that may be combined with any of the preceding embodiments, the at least one antibody that specifically binds to an inhibitory checkpoint molecule is selected from the group consisting of an anti-PD-L1 antibody, an anti-CTLA4 antibody, an anti-PD-L2 antibody, an anti-PD-1 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, and anti-HVEM antibody, an anti- B- and T-lymphocyte attenuator (BTLA) antibody, an anti-Killer inhibitory receptor (KIR) antibody, an anti-GAL9 antibody, an anti-TIM3 antibody, an anti-A2AR antibody, an anti-LAG-3 antibody, an anti-phosphatidylinerine antibody, an anti-CD27 antibody, and any combination thereof. In certain embodiments that may be combined with any of the preceding embodiments, the standard or investigational anti-cancer therapy is one or more therapies selected from the group consisting of radiotherapy, cytotoxic chemotherapy, targeted therapy, imatinib (Gleevec®), trastuzumab (Herceptin®), adoptive cell transfer (ACT), chimeric antigen receptor T cell transfer (CAR-T), vaccine therapy, and cytokine therapy. In certain embodiments that may be combined with any of the preceding embodiments, the method further comprises administering to the individual at least one antibody that specifically binds to an inhibitory cytokine. In certain embodiments that may be combined with any of the preceding embodiments, the at least one antibody that specifically binds to an inhibitory cytokine is administered in combination with the isolated antibody. In certain embodiments that may be combined with any of the preceding embodiments, the at least one antibody that specifically binds to an inhibitory cytokine is selected from the group consisting of an anti-CCL2 antibody, an anti-CSF-1 antibody, an anti-IL-2 antibody, and any combination thereof. In certain embodiments that may be combined with any of the preceding embodiments, the method further comprises administering to the individual at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein. In certain embodiments that may be combined with any of the preceding embodiments, the at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein is administered in combination with the isolated antibody. In certain embodiments that may be combined with any of the preceding embodiments, the at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein is selected from the group consisting of an agonist anti-CD40 antibody, an
agonist anti-OX40 antibody, an agonist anti-ICOS antibody, an agonist anti-CD28 antibody, an agonist anti-CD137/4-1BB antibody, an agonist anti-CD27 antibody, an agonist anti-glucocorticoid-induced TNFR-related protein GITR antibody, and any combination thereof. In certain embodiments that may be combined with any of the preceding embodiments, the method further comprises administering to the individual at least one stimulatory cytokine. In certain embodiments that may be combined with any of the preceding embodiments, the at least one stimulatory cytokine is administered in combination with the isolated antibody. In certain embodiments that may be combined with any of the preceding embodiments, the at least one stimulatory cytokine is selected from the group consisting of TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL20 family member, IL-33, LIF, OSM, CNTF, TGF-beta, IL-11, IL-12, IL-17, IL-8, CRP, IFN-α, IFN-β, IL-2, IL-18, GM-CSF, G-CSF, and any combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] FIG. 1A shows an amino acid sequence alignment between the human TREM2 protein and the human NCTR2 protein, depicting the homology between the two proteins.

FIG. 1B shows a structure-based sequence alignment between several TREM proteins and other members of the IgV family. The amino acid residue numbering is consistent with the mature sequence of the human TREM1 protein. The secondary structure elements of TREM1 are illustrated as arrows for the β strands and cylinders for α helices. Amino acid residues involved in homo- and heterodimer formation are shown on black background. Cysteine residues that form disulfide bonds and that are conserved for the V-type Ig fold, are depicted in bold and marked with asterisks. Gaps are indicated by “—”. M-1 residues violating antibody-like dimer formation mode are marked with closed triangles as (e.g., Radaev et al., (2003) Structure. 11(12):1527-1535).

[0043] FIG. 2A shows an amino acid sequence alignment between the human TREM1 protein and the human TREM2 protein, depicting the homology between the two proteins.

FIG. 2B shows the amino acid sequences of the heavy chain variable regions of TREM2 antibodies. The CDR sequences are underlined in each sequence. Sequence regions in-between
each underlined CDR sequence correspond to the framework regions. **FIG. 2C** shows the amino acid sequences of the light chain variable regions of TREM2 antibodies. The CDR sequences are underlined in each sequence. Sequence regions in-between each underlined CDR sequence correspond to the framework regions.

**[0044]** **FIG. 3A** shows FACS histograms demonstrating binding of TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 to a mouse cell line (BWZ T2) expressing recombinant mouse TREM2. **FIG. 3B** shows antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 binding to WT (Trem +/+ ) and TREM2 deficient (TREM2 −/−) bone marrow derived mouse macrophages (BMMac). Antibody Ab88 represents the negative isotype control. Shaded histograms represent the TREM2 antibody negative population. Black outlined histograms represent the TREM2 antibody positive population.

**[0045]** **FIG. 4A** shows FACS histograms demonstrating binding of TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab43, Ab45, Ab60, and Ab65 to a human cell line (293) expressing recombinant human TREM2-DAP12 fusion protein. Shaded histograms represent a TREM2 antibody negative population. Black outlined histograms represent a TREM2 antibody positive population. **FIG. 4B** shows antibodies Ab1, Ab9, Ab14, Ab22, Ab43, Ab45, Ab60, and Ab65 binding to primary human dendritic cells (hDCs). Antibody Ab88 represents the negative isotype control. Shaded histograms represent secondary antibody alone negative control. Black outlined histograms represent the TREM2 antibody positive population.

**[0046]** **FIG. 5A** shows FACS dot plots demonstrating expression of cell surface markers CD83 and CD86 on human dendritic cells (DCs) after incubation with plate-bound TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65. Antibody Ab88 represents the negative isotype control. Plots were gated on CD11cH+ HLA-DRH+ LIN− DCs. Percentage of cells within the CD83+CD86+ gate is displayed on each plot. **FIG. 5B** shows FACS histograms demonstrating expression of cell surface marker CD86 on human dendritic cells (DCs) after incubation with cross-linked TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65.
Antibodies were cross-linked with anti-human secondary antibody. Antibody Ab88 represents the negative isotype control.

[0047] FIG. 6A shows protein levels of inflammatory cytokines TNFa, IL-6, IL-10, and MCP-1 secreted in response to stimulation of WT and TREM2 KO macrophages with inflammatory mediators LPS or Zymosan. FIG. 6B shows protein levels of inflammatory cytokines IL-6 and TNFa secreted in response to stimulation of WT, TREM2 heterozygous (Het), and TREM2 KO macrophages with the cytokines IL-4 or IFN-γ.

[0048] FIG. 7A shows Syk phosphorylation as determined by western blot in wild-type and TREM2 deficient (Trem2−/−) mouse macrophages after incubation with TREM2 antibodies Ab1, Ab9, or Ab45. Antibodies Ab89 and Ab92 are negative isotype controls. FIG. 7B shows Syk phosphorylation as determined by western blot in human macrophages after incubation with TREM2 antibodies Ab1, Ab9, Ab14, Ab20, Ab22, Ab45, and Ab65. Antibodies Ab16 and Ab77 are non-agonistic negative controls. FIG. 7C shows Syk phosphorylation as determined by western blot in primary human dendritic cells after incubation with TREM2 antibodies Ab1, Ab5, Ab9, Ab22, Ab45, or Ab65.

FIG. 8A shows numbers of live WT, TREM2 heterozygous (TREM2+/−), and TREM2 KO (TREM2−/−) macrophages after culture in growth factor M-CSF for the indicated number of days. FIG. 8B shows FACS plots demonstrating DAPI staining of WT, TREM2 heterozygous (TREM2+/−), and TREM2 KO (TREM2−/−) macrophages after culture in M-CSF for 6 days (+M-CSF) or in M-CSF for 4 days, followed by no M-CSF for 36 hours (-M-CSF). Percentage of live macrophages within the CD11b+DAPI- gate is indicated on each plot. FIG. 8C shows luminescence levels detected in a luciferase viability assay after culture of WT and TREM2 KO dendritic cells, M1 macrophages, and M2 macrophages in growth factors GM-CSF, M-CSF, or M-CSF+IL-4, respectively.

[0049] FIG. 9 shows competitive binding between TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, Ab65, Ab66, and Ab68 and E. coli cells expressing putative TREM2 ligand to mouse and human cell lines expressing TREM2. Bacterial binding is expressed as a percentage
of control. Average of two independent experiments; black bars: no difference to isotype control, red bars: significantly different from isotype controls (ANOVA).

[0050] FIG. 10A shows DAP12 phosphorylation as determined by western blot in mouse macrophages after incubation with TREM2 antibody Ab45 or Ab65. FIG. 10B shows DAP12 phosphorylation as determined by western blot in wild-type and TREM2 deficient (Trem2/-) mouse macrophages after incubation with TREM2 antibody Ab1, Ab9, Ab22, or Ab45.

[0051] FIG. 11A shows FACS data demonstrating expression of cell surface markers CD86 and CD206 on WT, TREM2 heterozygous (Het), and TREM2 KO macrophages after stimulation with the cytokines IL-4 or IFN-γ. FIG. 11B shows expression of cell surface marker CD86 on WT and TREM2 KO macrophages after stimulation with the inflammatory mediators LPS or Zymosan.

[0052] FIG. 12 shows phagocytosis of apoptotic cells and E. coli by wild-type (WT) and TREM2 KO (TREM2/-) bone marrow derived macrophages (BMMacs) cultured without MCSF.

[0053] FIG. 13A shows an epitope map of TREM2 antibodies Ab1 and Ab9. FIG. 13B shows an epitope map of TREM2 antibodies Ab45 and Ab65.

[0054] FIG. 14 shows Fortebio analysis demonstrating simultaneous binding of antibody MAB17291 and antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 to TREM2-Fc. Control antibodies Ab63 and Ab87 did not simultaneously bind to TREM2-Fc.

[0055] FIG. 15 shows the percent increased survival of wild-type (WT) and TREM2 knock-out (KO) mouse bone marrow derived macrophages cultured in the presence of plate bound, cross-linked TREM2 antibody Ab22, Ab45, or Ab65 Fabs and M-CSF. Antibody Ab88 represents the negative isotype control.

[0056] FIG. 16A shows the luminescence of mouse bone marrow derived macrophages cultured in the presence of soluble, non-cross-linked TREM2 antibody Fabs and M-CSF. Antibody Ab99 represents the negative isotype control. FIG. 16B shows the luminescence of
mouse bone marrow derived macrophages cultured in the presence of soluble, full-length TREM2 antibodies and M-CSF. Antibody Ab91 represents the negative isotype control. The “NT” dotted line indicates the average viability obtained with untreated macrophages (no antibody added). The “no MCSF” dotted line indicates the average viability obtained when macrophages are cultured in the absence of M-CSF.

[0057] FIG. 17A shows induction of TREM2-dependent gene expression by plate bound, full-length anti-TREM2 antibodies using a luciferase reporter gene in a cell-based assay. FIG. 17B shows induction of TREM2-dependent gene expression by plate bound, full-length anti-TREM2 antibodies using a luciferase reporter gene in a cell-based assay. FIG. 17C shows induction of TREM2-dependent gene expression by plate bound phosphatidylserine (PS).

[0058] FIG. 18 shows inhibition of TREM2-dependent gene expression by soluble, full-length anti-TREM2 antibodies using a luciferase reporter gene in a cell-based assay.

DETAILED DESCRIPTION OF THE INVENTION

General techniques


**Definitions**

[0060] As used herein, the term “preventing” includes providing prophylaxis with respect to occurrence or recurrence of a particular disease, disorder, or condition in an individual. An individual may be predisposed to, susceptible to a particular disease, disorder, or condition, or at risk of developing such a disease, disorder, or condition, but has not yet been diagnosed with the disease, disorder, or condition.

[0061] As used herein, an individual “at risk” of developing a particular disease, disorder, or condition may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. “At risk” denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of a particular disease, disorder, or condition, as known in the art. An individual having one or more of these risk factors has a higher probability of developing a particular disease, disorder, or condition than an individual without one or more of these risk factors.

[0062] As used herein, the term “treatment” refers to clinical intervention designed to alter the natural course of the individual being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of progression, ameliorating or
palliating the pathological state, and remission or improved prognosis of a particular disease, disorder, or condition. An individual is successfully “treated”, for example, if one or more symptoms associated with a particular disease, disorder, or condition are mitigated or eliminated.

[0063] An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. An effective amount can be provided in one or more administrations.

[0064] A “therapeutically effective amount” is at least the minimum concentration required to effect a measurable improvement of a particular disease, disorder, or condition. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the anti-TREM2 antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the anti-TREM2 antibody are outweighed by the therapeutically beneficial effects.

[0065] As used herein, administration “in conjunction” with another compound or composition includes simultaneous administration and/or administration at different times. Administration in conjunction also encompasses administration as a co-formulation or administration as separate compositions, including at different dosing frequencies or intervals, and using the same route of administration or different routes of administration.

[0066] An “individual” for purposes of treatment, prevention, or reduction of risk refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sport, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, and the like. Preferably, the individual is human.

[0067] The term “immunoglobulin” (Ig) is used interchangeably with “antibody” herein. The term “antibody” herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.
The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. The pairing of a $V_H$ and $V_L$ together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., *Basic and Clinical Immunology*, 8th Ed., Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha ("α"), delta ("δ"), epsilon ("ε"), gamma ("γ") and mu ("μ"), respectively. The γ and α classes are further divided into subclasses (isotypes) on the basis of relatively minor differences in the CH sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The subunit structures and three dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al., *Cellular and Molecular Immunology*, 4th ed. (W.B. Saunders Co., 2000).

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain ($V_H$) followed by a number of constant domains. Each light chain has a variable domain at one end ($V_L$) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.
An “isolated” antibody, such as an isolated anti-TREM2 antibody of the present disclosure, is one that has been identified, separated and/or recovered from a component of its production environment (e.g., naturally or recombinantly). Preferably, the isolated polypeptide is free of association with all other contaminant components from its production environment. Contaminant components from its production environment, such as those resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant T-cells since at least one component of the antibody’s natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

The “variable region” or “variable domain” of an antibody, such as an anti-TREM2 antibody of the present disclosure, refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as “VH” and “VL”, respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

The term “variable” refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies, such as anti-TREM2 antibodies of the present disclosure. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely
adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent-cellular toxicity.

[0074] The term “monoclonal antibody” as used herein refers to an antibody, such as a monoclonal anti-TREM2 antibody of the present disclosure, obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (e.g., isomerizations, amidations, etc.) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler and Milstein., *Nature*, 256:495-97 (1975); Hongo et al., *Hybridoma*, 14 (3):253-260 (1995), Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2d ed. 1988); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage-display technologies (see, e.g., Clackson et al., *Nature*, 352:624-628 (1991); Marks et al., *J. Mol. Biol.* 222:581-597 (1992); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5):1073-1093.

[0075] The terms “full-length antibody,” “intact antibody” or “whole antibody” are used interchangeably to refer to an antibody, such as an anti-TREM2 antibody of the present disclosure, in its substantially intact form, as opposed to an antibody fragment. Specifically, whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0076] An “antibody fragment” comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')2 and Fv fragments; diabodies; linear antibodies (see U.S. Patent 5,641,870, Example 2; Zapata et al., *Protein Eng.* 8(10):1057-1062 (1995)); single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0077] Papain digestion of antibodies, such as anti-TREM2 antibodies of the present disclosure, produces two identical antigen-binding fragments, called “Fab” fragments, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (CH1). Each Fab fragment is monovalent with
respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')$_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C$_{H1}$ domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')$_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0078] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0079] “Fv” is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0080] “Single-chain Fv” also abbreviated as “sFv” or “scFv” are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V$_H$ and V$_L$ domains, which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).
“Functional fragments” of antibodies, such as anti-TREM2 antibodies of the present disclosure, comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the F region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

The term “diabodies” refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the VH and VL domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, i.e., a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two “crossover” sFv fragments in which the VH and VL domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger et al., Proc. Nat’l Acad. Sci. USA 90:6444-48 (1993).

As used herein, a “chimeric antibody” refers to an antibody (immunoglobulin), such as a chimeric anti-TREM2 antibody of the present disclosure, in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Nat’l Acad. Sci. USA, 81:6851-55 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with an antigen of interest. As used herein, “humanized antibody” is used a subset of “chimeric antibodies.”

“Humanized” forms of non-human (e.g., murine) antibodies, such as humanized forms of anti-TREM2 antibodies of the present disclosure, are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized
antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, and the like. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, e.g., Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). *See also*, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.

[0085] A "human antibody" is one that possesses an amino-acid sequence corresponding to that of an antibody, such as an anti-TREM2 antibody of the present disclosure, produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J.*
ImmunoL., 147(1):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e.g., immunized xenomice (see, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li et al., Proc. Nat’l Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0086] The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody-variable domain, such as that of an anti-TREM2 antibody of the present disclosure, that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu et al., Immunity 13:37-45 (2000); Johnson and Wu in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, e.g., Hamers-Casterman et al., Nature 363:446-448 (1993) and Sheriff et al., Nature Struct. Biol. 3:733-736 (1996).

[0087] A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., supra). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody-modeling software. The “contact” HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

<table>
<thead>
<tr>
<th>Loop</th>
<th>Kabat</th>
<th>AbM</th>
<th>Chothia</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>L24-L34</td>
<td>L24-L34</td>
<td>L26-L32</td>
<td>L30-L36</td>
</tr>
</tbody>
</table>

sf-3499172
HVRs may comprise “extended HVRs” as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 or 89-96 (L3) in the VL, and 26-35 (H1), 50-65 or 49-65 (a preferred embodiment) (H2), and 93-102, 94-102, or 95-102 (H3) in the VH. The variable-domain residues are numbered according to Kabat et al., supra, for each of these extended-HVR definitions.

“Framework” or “FR” residues are those variable-domain residues other than the HVR residues as herein defined.

The phrase “variable-domain residue-numbering as in Kabat” or “amino-acid-position numbering as in Kabat,” and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat et al., supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g., residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service,
National Institutes of Health, Bethesda, Md. (1991)). The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., supra). The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody. References to residue numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. References to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system (e.g., see United States Patent Publication No. 2010-280227).

[0092] An “acceptor human framework” as used herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. Where pre-existing amino acid changes are present in a VH, preferable those changes occur at only three, two, or one of positions 71H, 73H and 78H; for instance, the amino acid residues at those positions may by 71A, 73T and/or 78A. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0093] A “human consensus framework” is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat et al., supra. Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat et al., supra.
An “amino-acid modification” at a specified position, e.g., of an anti-TREM2 antibody of the present disclosure, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion “adjacent” to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. The preferred amino acid modification herein is a substitution.

An “affinity-matured” antibody, such as an affinity matured anti-TREM2 antibody of the present disclosure, is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al., Bio/Technology 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of HVR and/or framework residues is described by, for example: Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147-155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

As use herein, the term “specifically recognizes” or “specifically binds” refers to measurable and reproducible interactions such as attraction or binding between a target and an antibody, such as between an anti-TREM2 antibody and TREM2 that is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody, such as an anti-TREM2 antibody of the present disclosure, that specifically or preferentially binds to a target or an epitope is an antibody that binds this target or epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets or other epitopes of the target. It is also understood by reading this definition that, for example, an antibody (or a moiety) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. An antibody that specifically binds to a target may
have an association constant of at least about $10^3$ M$^{-1}$ or $10^4$ M$^{-1}$, sometimes about $10^5$ M$^{-1}$ or $10^6$ M$^{-1}$, in other instances about $10^6$ M$^{-1}$ or $10^7$ M$^{-1}$, about $10^8$ M$^{-1}$ to $10^9$ M$^{-1}$, or about $10^{10}$ M$^{-1}$ to $10^{11}$ M$^{-1}$ or higher. A variety of immunoassay formats can be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

[0097] As used herein, an “interaction” between a TREM2 protein, or DAP12 protein, and a second protein encompasses, without limitation, protein-protein interaction, a physical interaction, a chemical interaction, binding, covalent binding, and ionic binding. As used herein, an antibody “inhibits interaction” between two proteins when the antibody disrupts, reduces, or completely eliminates an interaction between the two proteins. An antibody of the present disclosure, or fragment thereof, “inhibits interaction” between two proteins when the antibody or fragment thereof binds to one of the two proteins.

[0098] An “agonist” antibody or an “activating” antibody is an antibody, such as an agonist anti-TREM2 antibody of the present disclosure, that induces (e.g., increases) one or more activities or functions of the antigen after the antibody binds the antigen.

[0099] An “antagonist” antibody or a “blocking” antibody is an antibody, such as an antagonist anti-TREM2 antibody of the present disclosure, that reduces or eliminates (e.g., decreases) antigen binding to one or more ligand after the antibody binds the antigen, and/or that reduces or eliminates (e.g., decreases) one or more activities or functions of the antigen after the antibody binds the antigen.

[0100] Antibody “effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype.

[0101] The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions.
Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the invention include human IgG1, IgG2, IgG3 and IgG4.

[0102] A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[0103] A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

[0104] “Fc receptor” or “FcR” describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII,
and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (“ITAM”) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (“ITIM”) in its cytoplasmic domain. (see, e.g., M. Daëron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol. 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. FcRs can also increase the serum half-life of antibodies.

[0105] Binding to FcRn in vivo and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. See also, e.g., Shields et al., J. Biol. Chem. 9(2):6591-6604 (2001).

[0106] As used herein, “percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence refers to the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms known in the art needed to achieve maximal alignment over the full-length of the sequences being compared.
[0107] An “isolated” nucleic acid molecule encoding an antibody, such as an anti-TREM2 antibody of the present disclosure, is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0108] The term “vector,” as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double stranded DNA into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors,” or simply, “expression vectors.” In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector.

[0109] “Polynucleotide,” or “nucleic acid,” as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be
interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, “caps,” substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomerically nucleic acids, etc.), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5’ and 3’ terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2’-O-methyl-, 2’-O-allyl-, 2’-fluoro- or 2’-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S (“thioate”), P(S)S (“thioate”), (O)NR2 (“amidate”), P(O)R, P(O)OR’, CO, or CH2 (“formacetal”), in which each R or R’ is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkanyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0110] A “host cell” includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a
single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected \textit{in vivo} with a polynucleotide(s) of this invention.

\textbf{[0111]} \hspace{0.2cm} \textit{Carriers} as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

\textbf{[0112]} The term \textit{about} as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to \textit{about} a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter \textit{per se}.

\textbf{[0113]} As used herein and in the appended claims, the singular forms \textit{a}, \textit{an}, and \textit{the} include plural reference unless the context clearly indicates otherwise. For example, reference to an \textit{antibody} is a reference to from one to many antibodies, such as molar amounts, and includes equivalents thereof known to those skilled in the art, and so forth.

\textbf{[0114]} It is understood that aspect and embodiments of the invention described herein include \textit{comprising}, \textit{consisting}, and \textit{consisting essentially of} aspects and embodiments.

\textbf{Overview}

\textbf{[0115]} The present disclosure relates to anti-TREM2 antibodies with one or more agonist or antagonist activities; methods of making and using such antibodies; pharmaceutical compositions
containing such antibodies; nucleic acids encoding such antibodies; and host cells containing nucleic acids encoding such antibodies.

[0116] In some embodiments, and without wishing to be bound by theory, it is believed that the agonistic activities of the anti-TREM2 antibodies of the present disclosure are due, at least in part, to the ability of the antibodies to induce or retain TREM2 receptor clustering on the surface of cells. In some embodiments, it is believed that anti-TREM2 antibodies can induce or retain TREM2 receptor clustering in vivo by not only binding specifically to TREM2, but by also binding to Fc receptors on adjacent cells, which leads to antibody aggregation that in turn aggregates TREM2. Advantageously, certain immunoglobulin isotypes, including without limitation, IgG2 and IgM, have an intrinsic ability to induce or retain clustering of target antigens (e.g., TREM2) without binding Fc receptors on adjacent cells. In some embodiments, agonistic TREM2 activities can be determined or tested in vitro by any of several techniques disclosed herein (see, e.g., Examples 3, 5, 9, 25, 27, 37 and 39), including, without limitation, plate-binding full-length anti-TREM2 antibodies to increase the density of antibodies exposed to TREM2 and cross-linking anti-TREM2 antibodies.

[0117] Accordingly, certain aspects of the present disclosure are based, at least in part, on the identification of anti-TREM2 antibodies that are capable of binding to both human and mouse TREM2 with high affinity (see, e.g., Example 1); that compete with TREM2-ligand for binding to the ligand-binding site on human and mouse TREM2 (see, e.g., Example 25); and that exhibit one or more agonistic TREM2 activities, including, without limitation, induction of CD83+CD86+ dendritic cells (see, e.g., Example 3), inhibition of inflammatory cytokine secretion (see, e.g., Example 5); induction of the TREM2 downstream signaling molecule Syk in macrophages and dendritic cells (see, e.g., Example 9), induction of the TREM2 signaling adaptor molecule DAP12 in macrophages (see, e.g., Example 27), induction of cell survival of innate immune cells, such as macrophages (see, e.g., Example 37), and activation of TREM2-dependent gene expression (see, e.g., Examples 39 and 41).

[0118] Further aspects of the present disclosure are based, at least in part, on the surprising discovery that the anti-TREM2 antibodies of the present disclosure can also induce antagonistic
activities when the antibody is produced or otherwise formatted such that it is incapable of inducing or retaining TREM2 receptor clustering. In some embodiments, anti-TREM2 antibodies of the present disclosure exhibit one or more antagonistic TREM2 activities, including, without limitation, inhibition of cell survival of innate immune cells ((see, e.g., Example 38), and inhibition of TREM2-dependent gene expression (see, e.g., Example 40).

**TREM2 proteins**

[0119] In one aspect, the invention provides antibodies that bind to a TREM2 protein of the present disclosure and modulate one or more TREM2 activities after binding to a TREM2 protein expressed in a cell.

[0120] TREM2 proteins of the present disclosure include, without limitation, a mammalian TREM2 protein, human TREM2 protein (Uniprot Accession No. Q9NZC2), mouse TREM2 protein (Uniprot Accession No. Q9NH8), rat TREM2 protein (Uniprot Accession No. D3ZZ89), Rhesus monkey TREM2 protein (Uniprot Accession No. F6QVF2), bovine TREM2 protein (Uniprot Accession No. Q05B59), equine TREM2 protein (Uniprot Accession No. F7D6L0), pig TREM2 protein (Uniprot Accession No. H2EZZ3), and dog TREM2 protein (Uniprot Accession No. E2RP46). As used herein “TREM2 protein” refers to both wild-type sequences and naturally occurring variant sequences.

[0121] Triggering receptor expressed on myeloid cells-2 (TREM2) is variously referred to as TREM-2, Trem2a, Trem2b, Trem2c, triggering receptor expressed on myeloid cells-2a, and triggering receptor expressed on monocytes-2. TREM2 is a 230 amino acid membrane protein. TREM2 is an immunoglobulin-like receptor primarily expressed on myeloid lineage cells, including without limitation, macrophages, dendritic cells, monocytes, Langerhans cells of skin, Kupffer cells, osteoclasts, and microglia. In some embodiments, TREM2 forms a receptor signaling complex with DAP12. In some embodiments, TREM2 phosphorylates and signals through DAP12 (an ITAM domain adaptor protein). In some embodiments TREM2 signaling results in the downstream activation of PI3K or other intracellular signals. On Myeloid cells, Toll-like receptor (TLR) signals are important for the activation of TREM2 activities, e.g., in the
context of an infection response. TLRs also play a key role in the pathological inflammatory response, e.g., TLRs expressed in macrophages and dendritic cells.

[0122] In some embodiments, an example of a human TREM2 amino acid sequence is set forth below as SEQ ID NO: 1:

```
10  MEPLRLILLL FVTELSGAHN TTVFQGAVGQ SLQVSCPYDS MKHGRPRKAW CRQLGEKGP
20  CQVVSHTMLS LGNFRMRWNG STAITDDTLG GILITTRNLQ QPDAGLYQC QSLHGSEADT
30  LRKVLVEVLA DPLDHRAAGD LWFGESESF EDARHVEHIS RSLLEEGIPF PPTSLLLLA
40  190  CIFIKLILAA SAWAAAMHG QKPGTHPPSE LCDCGHDPGYQ LQTLPGLRDT
45  200  210  220  230
```

[0123] In some embodiments, the human TREM2 is a preprotein that includes a signal peptide. In some embodiments, the human TREM2 is a mature protein. In some embodiments, the mature TREM2 protein does not include a signal peptide. In some embodiments, the mature TREM2 protein is expressed on a cell. In some embodiments, TREM2 contains a signal peptide located at amino acid residues 1-18 of human TREM2 (SEQ ID NO: 1); an extracellular immunoglobulin-like variable-type (IgV) domain located at amino acid residues 29-112 of human TREM2 (SEQ ID NO: 1); additional extracellular sequences located at amino acid residues 113-174 of human TREM2 (SEQ ID NO: 1); a transmembrane domain located at amino acid residues 175-195 of human TREM2 (SEQ ID NO: 1); and an intracellular domain located at amino acid residues 196-230 of human TREM2 (SEQ ID NO: 1).

[0124] The transmembrane domain of human TREM2 contains a lysine at amino acid residue 186 that can interact with an aspartic acid in DAP12, which is a key adaptor protein that transduces signaling from TREM2, TREM1, and other related IgV family members.

[0125] Homologues of human TREM2 include, without limitation, the natural killer (NK) cell receptor NK-p44 (NCTR2), the polymeric immunoglobulin receptor (pIgR), CD300E, CD300A, CD300C, and TREML1/TLT1. In some embodiments, NCTR2 has similarity with TREM2 within the IgV domain.
DAP12 proteins

[0126] In one aspect, the invention provides antibodies that bind to a DAP12 protein of the present disclosure and modulate one or more DAP12 activities after binding to a DAP12 protein expressed in a cell.

[0127] DAP12 proteins of the present disclosure include, without limitation, a mammalian DAP12 protein, human DAP12 protein (Uniprot Accession No. O43914), mouse DAP12 protein (Uniprot Accession No. O54885), rat DAP12 protein (Uniprot Accession No. Q6X9T7), Rhesus monkey DAP12 protein (Uniprot Accession No. Q8WNQ8), bovine DAP12 protein (Uniprot Accession No. Q95J80), and pig DAP12 protein (Uniprot Accession No. Q9TU45). As used herein “DAP12 protein” refers to both wild-type sequences and naturally occurring variant sequences.

[0128] DNAX-activation protein 12 (DAP12) is variously referred to as Killer-activating receptor-associated protein, KAR-associated protein (KARAP), PLOSL, PLO-SL, TYRO protein, and tyrosine kinase-binding protein. DAP12 is a 113 amino acid membrane protein. In some embodiments, DAP12 functions as a transmembrane signaling polypeptide, which contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. It may associate with the killer-cell inhibitory receptor (KIR) family of membrane glycoproteins and may act as an activating signal transduction element. In other embodiments, the DAP12 protein may bind zeta-chain (TCR) associated protein kinase 70kDa (ZAP-70) and spleen tyrosine kinase (SYK), and play a role in signal transduction, bone modeling, brain myelination, and inflammation.

[0129] Mutations within the DAP12-encoding gene have been associated with polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), also known as Nasu-Hakola disease. Without wishing to be bound by theory, it is believed that the DAP12 receptor is TREM2, which also causes PLOSL. Multiple alternative transcript variants encoding distinct isoforms of DAP12 have been identified. DAP12 non-covalently associates with activating receptors of the CD300 family. Cross-linking of CD300-TYROBP/DAP12 complexes results in cellular activation, such as neutrophil activation mediated by integrin. DAP12 is a
homodimer; disulfide-linked protein. In some embodiments, DAP12 interacts with SIRPB1, TREM1, CLEC5F, SIGLEC14, CD300LB, CD300E, and CD300D by similarity and via ITAM domain, as well as with SYK via SH2 domain. In other embodiments, DAP12 activates SYK, which mediates neutrophils and macrophages integrin-mediated activation. In other embodiments, DAP12 interacts with KLRC2 and KIR2DS3.

[0130] In some embodiments, an example of a human DAP12 amino acid sequence is set forth below as SEQ ID NO: 2:

```
10  MGGLFCSRL LLLFLLAVS GLRPVQAQQ SDCSCSTVSP GVLAGIVMGL LVLTVLIALA
20  VYFLGRLVPR GRGAAEAATR KQRITETESP YQELQGQRSD VYSDLNTQRP YYK
```

[0131] In some embodiments, the human DAP12 is a preprotein that includes a signal peptide. In some embodiments, the human DAP12 is a mature protein. In some embodiments, the mature DAP12 protein does not include a signal peptide. In some embodiments, the mature DAP12 protein is expressed on a cell. DAP12 is a single-pass type I membrane protein. It contains an extracellular domain located at amino acid residues 22-40 of human DAP12 (SEQ ID NO: 2); a transmembrane domain located at amino acid residues 41-61 of human DAP12 (SEQ ID NO: 2); and an intracellular domain located at amino acid residues 62-113 of human DAP12 (SEQ ID NO: 2). The immunoreceptor tyrosine-based activation motif (ITAM) domain is located at amino acid residues 80-118 of human DAP12 (SEQ ID NO: 2).

[0132] In some embodiments, an aspartic acid residue in DAP12 interacts with the transmembrane domain of human TREM2 containing a lysine at amino acid residue 186, and transduces signaling from TREM2, TREM1, and other related IgV family member proteins.

**Anti-TREM2 antibodies**

[0133] Certain aspects of the present disclosure relate to antibodies that specifically bind to TREM2. In some embodiments, antibodies of the present disclosure bind a mature TREM2 protein. In some embodiments, antibodies of the present disclosure bind a mature TREM2 protein, wherein the mature TREM2 protein is expressed on a cell. In some embodiments,
antibodies of the present disclosure bind a TREM2 protein expressed on one or more human cells selected from human dendritic cells, human macrophages, human monocytes, human osteoclasts, human Langerhans cells of skin, human Kupffer cells, human microglia, and any combinations thereof. In some embodiments, antibodies of the present disclosure are agonist antibodies. In some embodiments, antibodies of the present disclosure are inert antibodies. In some embodiments, antibodies of the present disclosure are antagonist antibodies.

_Agonist anti-TREM2 antibodies_

**[0134]** Anti-TREM2 antibodies of the present disclosure generally bind to one or more TREM2 proteins expressed in a cell. One class of antibodies is agonist antibodies. For example, the TREM2 receptor is thought to require clustering on the cell surface in order to transduce a signal. Thus agonist antibodies may have unique features to stimulate, for example, the TREM2 receptor. For example, they may have the correct epitope specificity that is compatible with receptor activation, as well as the ability to induce or retain receptor clustering on the cell surface.

**[0135]** _In vivo_, antibodies may cluster receptors by multiple potential mechanisms. Some isotypes of human antibodies such as IgG2 have, due to their unique structure, an intrinsic ability to cluster receptors, or retain receptors in a clustered configuration, thereby activating receptors such as TREM2 without binding to an Fc receptor (e.g., White et al., (2015) Cancer Cell 27, 138–148).

**[0136]** Other antibodies cluster receptors (e.g., TREM2) by binding to Fcg receptors on adjacent cells. Binding of the constant IgG Fc part of the antibody to Fcg receptors leads to aggregation of the antibodies, and the antibodies in turn aggregate the receptors to which they bind through their variable region (Chu et al (2008) Mol Immunol, 45:3926-3933; and Wilson et al., (2011) Cancer Cell 19, 101–113). Binding to the inhibitory Fcg receptor FcgR (FcgRIIB) that does not elicit cytokine secretion, oxidative burst, increased phagocytosis, and enhanced antibody-dependent, cell-mediated cytotoxicity (ADCC) is often a preferred way to cluster antibodies _in vivo_, since binding to FcgRIIB is not associated with immune adverse effects.
[0137] Other mechanisms may also be used to cluster receptors (e.g., TREM2). For example, antibody fragments (e.g., Fab fragments) that are cross-linked together may be used to cluster receptors (e.g., TREM2) in a manner similar to antibodies with Fc regions that bind Fcg receptors, as described above. Without wishing to be bound to theory, it is thought that cross-linked antibody fragments (e.g., Fab fragments) may function as agonist antibodies if they induce receptor clustering on the cell surface and bind an appropriate epitope on the target (e.g., TREM2).

[0138] Therefore, in some embodiments, antibodies that bind a TREM2 protein may include agonist antibodies that due to their epitope specificity bind TREM2 and activate one or more TREM2 activities. Without wishing to be bound to theory, such antibodies may bind to the ligand-binding site on TREM2 and mimic the action of a natural ligand, or stimulate the target antigen to transduce signal by binding to one or more domains that are not the ligand-binding sites. Such antibodies would not interfere with ligand binding and may act additively or synergistically with the natural ligands.

[0139] In some embodiments, an antibody of the present disclosure is an agonist antibody that induces one or more TREM2 activities. In some embodiments the antibody induces one or more activities of TREM2 after binding to a TREM2 protein that is expressed in a cell. In certain embodiments the TREM2 protein is expressed on a cell surface. The TREM2 activities induced by anti-TREM2 antibodies of the present disclosure may include, without limitation, TREM2 autophosphorylation; DAP12 phosphorylation; Syk phosphorylation; recruitment of Syk, ZAP70, or both to a DAP12/TREM2 complex; PI3K activation; increased expression of cytokines; reduced expression of pro-inflammatory mediators; ERK phosphorylation; increased expression of CCR7; induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells; maturation of bone marrow-derived dendritic cells; enhancement or normalization of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation; increased ability of dendritic cells, monocytes, microglia, and/or macrophages to induce T-cell proliferation; induction of osteoclast production; increased rate of osteoclastogenesis, or both; increased survival and function of microglial cells (such as M1 microglial cells, activated M1 microglial cells, and/or M2 microglial cells), dendritic cells,
monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or macrophages (such as M1 macrophages, activated M1 macrophages, and/or M2 macrophages); resistance to apoptosis of microglial cells (such as M1 microglial cells, activated M1 microglial cells, and/or M2 microglial cells), dendritic cells, and/or macrophages (such as M1 macrophages, activated M1 macrophages, and/or M2 macrophages); induction of one or more types of clearance (such as apoptotic neuron clearance, nerve tissue debris clearance, non-nerve tissue debris clearance, bacteria or other foreign body clearance, disease-causing protein clearance, and tumor cell clearance); induction of phagocytosis of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, disease-causing proteins, disease-causing peptides, disease-causing nucleic acid (e.g., antisense GGCCCC (G2C4) repeat-expansion RNA), or tumor cells; reduced secretion of one or more inflammatory cytokines (e.g., FN-a4, IFN-b, IL-1β, TNF-α, IL-10, IL-6, IL-8, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, CRP, and MCP-1); reduced expression of one or more inflammatory receptors (such as CD86); increased phagocytosis by macrophages, dendritic cells, monocytes, and/or microglial cells (e.g., microglia) under conditions of reduced levels of MCSF; reduced phagocytosis by macrophages, dendritic cells, monocytes, and/or microglial cells in the presence of normal levels of MCSF; normalization of disrupted TREM2/DAP12-dependent gene expression; and increased activity of one or more TREM2-dependent genes (e.g., transcription factors of the nuclear factor of activated T-cells (NFAT) family of transcription factors). The anti-TREM2 antibodies of the present disclosure can be used to prevent, reduce risk of, or treat dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma, lupus, acute and chronic colitis, wound healing, Crohn’s disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet’s disease, Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear palsy, cortical basal ganglionic degeneration, acute disseminated encephalomyelitis, granulomatous disorders, Sarcoïdosis, diseases of aging, seizures, spinal cord injury, traumatic
brain injury, age related macular degeneration, glaucoma, retinitis pigmentosa, retinal
degeneration, respiratory tract infection, sepsis, eye infection, systemic infection, lupus, arthritis,
multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrosic disease, Paget's
disease of bone, and cancer. The anti-TREM2 antibodies of the present disclosure may also be
used in advanced wound care. In some embodiments, the anti-TREM2 antibodies of the present
disclosure are monoclonal antibodies. Anti-TREM2 antibodies of the present disclosure may be
tested for inducing one or more TREM2 activities (e.g., TREM2 autophosphorylation; DAP12
phosphorylation; Syk phosphorylation; recruitment of Syk, ZAP70, or both to a DAP12/TREM2
complex; PI3K activation; increased expression of cytokines; reduced expression of pro-
inflammatory mediators; ERK phosphorylation; increased expression of CCR7; induction of
microglial cell chemotaxis toward CCL19 and CCL21 expressing cells; maturation of bone
marrow-derived dendritic cells; enhancement or normalization of the ability of bone marrow-
derived dendritic cells to induce antigen-specific T-cell proliferation; increased ability of
dendritic cells, monocytes, microglia, and/or macrophages to induce T-cell proliferation;
induction of osteoclast production, increased rate of osteoclastogenesis, or both; increased
survival and function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells
of skin, Kupffer cells, and/or microglia; induction of one or more types of clearance; induction of
phagocytosis of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris,
bacteria, other foreign bodies, disease-causing proteins, disease-causing peptides, disease-causing
nucleic acid, or tumor cells; reduced secretion of one or more inflammatory cytokines; reduced
expression of one or more inflammatory receptors; increased phagocytosis by macrophages,
dendritic cells, monocytes, and/or microglial cells under conditions of reduced levels of MCSF;
reduced phagocytosis by macrophages, dendritic cells, monocytes, and/or microglial cells in the
presence of normal levels of MCSF; normalization of disrupted TREM2/DAP12-dependent gene
expression; and increased activity of one or more TREM2-dependent genes) using any suitable
method known in the art and/or described herein. For example, the anti-TREM2 antibodies can
be assayed in vitro for tyrosine phosphorylation of, TREM2, DAP12, Syk and/or ERK, by
assaying for recruitment of Syk and/or ZAP70 to DAP12, by assaying for PI3K activation, by
assaying for induction of expression of cytokines (e.g., IL-12p70, IL-6, and IL-10) or CCR7, or
by assaying for reduced expression of pro-inflammatory mediators (e.g., IL1-β and TNF) with TLR stimulation (e.g., LPS, CpG DNA, or Zymosan). Useful assays may include western blots (e.g., for tyrosine-phosphorylated DAP12 or threonine/serine-phosphorylated PI3K-kinase substrates), ELISA (e.g., for secreted interleukin or cytokine secretion), FACS (e.g., for anti-TREM2 binding to TREM2), immunocytochemistry (e.g., for e.g., for tyrosine-phosphorylated DAP12 or threonine/serine-phosphorylated PI3K-kinase substrates), reporter-gene assays (e.g., for TLR activation), increased survival and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia, increased phagocytosis of apoptotic neurons, damaged synapses, amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein Al, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, and proline-arginine (PR) repeat peptides, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, disease-causing proteins, disease-causing peptides, disease-causing nucleic acid, or tumor cells by macrophages, dendritic cells, Langerhans cells of skin, Kupffer cells, monocytes, osteoclasts, and/or microglial cells, increased cytoskeleton reorganization, and decreased microglial pro-inflammatory responses, or other assays known in the art.

[0140] An antibody dependent on binding to FcgR receptor to activate targeted receptors may lose its agonist activity if engineered to eliminate FcgR binding (see, e.g., Wilson et al., (2011) Cancer Cell 19, 101–113; Armour at al., (2003) Immunology 40 (2003) 585–593); and White et al., (2015) Cancer Cell 27, 138–148). As such, it is thought that an anti-TREM2 antibody of the present disclosure with the correct epitope specificity can be an agonist antibody and activate the target antigen, with minimal adverse effects, when the antibody has an Fc domain from a human IgG2 isotype (CH1 and hinge region) or another type of Fc domain that is capable of preferentially binding the inhibitory FcgRIIB receptors, or a variation thereof.
Exemplary agonist antibody Fc isotypes and modifications are provided in Table 2 below. In some embodiments, the agonist antibody has an Fc isotype listed in Table 2 below.

**Table 2. Exemplary anti-TREM2 agonist antibody Fc isotypes**

<table>
<thead>
<tr>
<th>Fc Isotype</th>
<th>Mutation (EU numbering scheme)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>N297A</td>
</tr>
<tr>
<td>IgG1</td>
<td>D265A and N297A</td>
</tr>
<tr>
<td>IgG1</td>
<td>L234A and L235A</td>
</tr>
<tr>
<td></td>
<td>L234A and G237A</td>
</tr>
<tr>
<td></td>
<td>L234A and L235A and G237A</td>
</tr>
<tr>
<td>IgG2</td>
<td>V234A and G237A</td>
</tr>
<tr>
<td>IgG4</td>
<td>L235A and G237A and E318A</td>
</tr>
<tr>
<td>IgG4</td>
<td>S228P and L236E</td>
</tr>
<tr>
<td>IgG2/4 hybrid</td>
<td>IgG2 aa 118-260 and IgG4 aa 261 to 447</td>
</tr>
<tr>
<td>IgG2</td>
<td>H268Q and V309L; and A330S and P331S</td>
</tr>
<tr>
<td>IgG1</td>
<td>C226S and C229S and E233P and L234V and L235A</td>
</tr>
<tr>
<td>IgG1</td>
<td>L234F and L235E and P331S</td>
</tr>
<tr>
<td>IgG2</td>
<td>C232S or C233S</td>
</tr>
<tr>
<td>IgG2</td>
<td>A330S and P331S</td>
</tr>
<tr>
<td>IgG1</td>
<td>S267E, and L328F</td>
</tr>
<tr>
<td></td>
<td>S267E alone</td>
</tr>
<tr>
<td>IgG2</td>
<td>S267E and L328F</td>
</tr>
<tr>
<td>IgG4</td>
<td>S267E and L328F</td>
</tr>
<tr>
<td>IgG2</td>
<td>WT HC with Kappa (light chain) LC</td>
</tr>
<tr>
<td></td>
<td>HC C127S with Kappa LC</td>
</tr>
<tr>
<td></td>
<td>Kappa LC C214S</td>
</tr>
<tr>
<td></td>
<td>Kappa LC C214S and HC C233S</td>
</tr>
<tr>
<td></td>
<td>Kappa LC C214S and HC C232S</td>
</tr>
<tr>
<td></td>
<td>Any of the above listed mutations together with P330S and P331S mutations</td>
</tr>
<tr>
<td></td>
<td>F(ab’)^2 fragment of WT IgG1 and any of the above listed mutations</td>
</tr>
<tr>
<td>IgG1</td>
<td>Substitute the Constant Heavy 1 (CH1) and hinge region of IgG1 With CH1 and hinge region of IgG2</td>
</tr>
<tr>
<td></td>
<td>ASTKGPSVFP LAPCSRSTSE STAALGCLVK</td>
</tr>
<tr>
<td></td>
<td>DYFPEPVTVS WNSGALTSGV HTFPAVLQSS</td>
</tr>
<tr>
<td></td>
<td>GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS</td>
</tr>
<tr>
<td></td>
<td>NTKVDKTVER KCCVECPCPC (SEQ ID NO: 21)</td>
</tr>
<tr>
<td></td>
<td>With a Kappa LC</td>
</tr>
<tr>
<td>Fc Isotype</td>
<td>Mutation (EU numbering scheme)</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>IgG1</td>
<td>Any of the above listed mutations together with A330L and/or L234F and/or L235E and/or P331S</td>
</tr>
<tr>
<td>IgG1, IgG2, or IgG4</td>
<td>Any of the above listed mutations together with M252Y and/or S254T and/or T256E</td>
</tr>
<tr>
<td>Mouse IgG1</td>
<td>For mouse disease models</td>
</tr>
<tr>
<td>IgG4</td>
<td>WT</td>
</tr>
</tbody>
</table>

[0142] In addition to the isotypes described in Table 2, and without wishing to be bound to theory, it is thought that antibodies with human IgG1 or IgG3 isotypes and mutants thereof (e.g., Strohl (2009) Current Opinion in Biotechnology 2009, 20:685–691) that bind the activating Fcg Receptors I, IIA, IIC, IIIA, IIIB in human and/or Fcg Receptors I, III and IV in mouse, may also act as agonist antibodies in vivo but may be associated with adverse effects related to ADCC. However, such Fcg receptors appear to be less available for antibody binding in vivo, as compared to the Inhibitory Fcg receptor FcgRIIB (see, e.g., White, et al., (2013) Cancer Immunol. Immunother. 62, 941–948; and Li et al., (2011) Science 333(6045):1030–1034.).

[0143] In some embodiments, the agonist antibody is of the IgG class, the IgM class, or the IgA class. In some embodiments, the agonist antibody has an IgG1, IgG2, IgG3, or IgG4 isotype.

[0144] In certain embodiments, the agonist antibody has an IgG2 isotype. In some embodiments, the agonist antibody contains a human IgG2 constant region. In some embodiments, the human IgG2 constant region includes an Fc region. In some embodiments, the agonist antibody induces the one or more TREM2 activities, the DAP12 activities, or both independently of binding to an Fc receptor. In some embodiments, the agonist antibody binds an inhibitory Fc receptor. In certain embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγIIIB). In some embodiments, the Fc region contains one or more modifications. For example, in some embodiments, the Fc region contains one or more amino acid substitutions (e.g., relative to a wild-type Fc region of the same isotype). In some embodiments, the one or more amino acid substitutions are selected from V234A (Alegre et al., (1994) Transplantation 57:1537-1543. 31; Xu et al., (2000) Cell Immunol, 200:16-26), G237A (Cole et al. (1999) Transplantation, 68:563-571), H268Q, V309L, A330S, P331S (US 2007/0148167; Armour et al. (1999) Eur J Immunol 29: 2613-2624; Armour et al. (2000) The

[0145] In some embodiments, the agonist antibody has an IgG2 isotype with a heavy chain constant domain that contains a C127S amino acid substitution, where the amino acid position is according to the EU numbering convention (White et al., (2015) Cancer Cell 27, 138-148; Lightle et al., (2010) PROTEIN SCIENCE 19:753-762; and WO2008079246).

[0146] In some embodiments, the agonist antibody has an IgG2 isotype with a Kappa light chain constant domain that contains a C214S amino acid substitution, where the amino acid position is according to the EU numbering convention (White et al., (2015) Cancer Cell 27, 138-148; Lightle et al., (2010) PROTEIN SCIENCE 19:753-762; and WO2008079246).

[0147] In certain embodiments, the agonist antibody has an IgG1 isotype. In some embodiments, the agonist antibody contains a mouse IgG1 constant region. In some embodiments, the agonist antibody contains a human IgG1 constant region. In some embodiments, the human IgG1 constant region includes an Fc region. In some embodiments, the agonist antibody binds an inhibitory Fc receptor. In certain embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγIIB). In some embodiments, the Fc region contains one or more modifications. For example, in some embodiments, the Fc region contains one or more amino acid substitutions (e.g., relative to a wild-type Fc region of the same isotype). In some embodiments, the one or more amino acid substitutions are selected from N297A (Bolt S et al. (1993) Eur J Immunol 23:403-411), D265A (Shields et al. (2001) R. J. Biol. Chem. 276, 6591–6604), L234A, L235A (Hutchins et al. (1995) Proc Natl Acad Sci USA, 92:11980-11984; Alegre et al., (1994) Transplantation 57:1537-1543, 31; Xu et al., (2000) Cell Immunol, 200:16-26), G237A (Alegre et al. (1994) Transplantation 57:1537-1543, 31; Xu et al. (2000) Cell Immunol, 200:16-26), C226S, C229S, E233P, L234V, L234F, L235E (McEarchern et al. (2007) Blood, 109:1185-1192), P331S (Sazinsky et al., (2008) Proc Natl Acad Sci USA 2008.

sf-3499172 -76-
105:20167-20172), S267E, L328F, A330L, M252Y, S254T, and/or T256E, where the amino acid position is according to the EU numbering convention.

[0148] In some embodiments, the antibody includes an IgG2 isotype heavy chain constant domain 1(CH1) and hinge region (White et al., (2015) Cancer Cell 27, 138–148). In certain embodiments, the IgG2 isotype CH1 and hinge region contain the amino acid sequence of ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFQTQTYTCNVDHKPSNTKVDKTVKCCVECPVP (SEQ ID NO:397). In some embodiments, the antibody Fc region contains a S267E amino acid substitution, a L328F amino acid substitution, or both, and/or a N297A or N297Q amino acid substitution, where the amino acid position is according to the EU numbering convention.

[0149] In certain embodiments, the agonist antibody has an IgG4 isotype. In some embodiments, the agonist antibody contains a human IgG4 constant region. In some embodiments, the human IgG4 constant region includes an Fc region. In some embodiments, the agonist antibody binds an inhibitory Fc receptor. In certain embodiments, the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγIIB). In some embodiments, the Fc region contains one or more modifications. For example, in some embodiments, the Fc region contains one or more amino acid substitutions (e.g., relative to a wild-type Fc region of the same isotype). In some embodiments, the one or more amino acid substitutions are selected from L235A, G237A, S228P, L236E (Reddy et al., (2000) J Immunol, 164:1925-1933), S267E, E318A, L328F, M252Y, S254T, and/or T256E, where the amino acid position is according to the EU numbering convention.

[0150] In certain embodiments, the agonist antibody has a hybrid IgG2/4 isotype. In some embodiments, the agonist antibody includes an amino acid sequence containing amino acids 118 to 260 according to EU numbering of human IgG2 and amino acids 261-447 according to EU numbering of human IgG4 (WO 1997/11971; WO 2007/106585).

In some embodiments, the Fc region further contains one or more additional amino acid substitutions selected from the group consisting of A330L, L234F, L235E, or P331S according to EU numbering; and any combination thereof.

**Inert antibodies**

Another class of antibodies of the present disclosure includes inert antibodies. As used herein, “inert” antibodies refer to antibodies that specifically bind their target antigen but do not modulate (e.g., decrease/inhibit or activate/induce) antigen function. For example, in the case of TREM2, inert antibodies do not modulate ligand binding and/or TREM2 activities. Without wishing to be bound to theory, it is thought that antibodies that do not have the ability to cluster TREM2 on the cell surface may be inert antibodies even if they have an epitope specificity that is compatible with receptor activation.

In some embodiments, antibodies that bind a TREM2 protein may include antibodies that bind TREM2 but, due to their epitope specificity, do not modulate protein function. Such functionally inert antibodies can be used as cargo to transport toxins or to tumor cells as described for the CD33 antibody Gemtuzumab zogamicin, (marketed as Mylotarg) which is conjugated to the cytotoxic agent from the class of calicheamicins and is used to target and kill acute myelogenous leukemia tumors (Naito et al., (2000), Leukemia, 14, 1436-1443; Ricart (2011) Clin Cancer Res 17; 6417-6436; Hamann et al., (2002) Journal: Bioconjugate Chemistry, 13, 47-58; and Beitz et al., (2001) Clin Cancer Res 7 ; 1490–6.). Therefore, in some embodiments, antibodies of the present disclosure are inert antibodies that bind TREM2 but are incapable of inducing one or more TREM2 activities (e.g., a TREM2 activity described herein).

Exemplary inert antibody Fc isotypes and modifications are provided in Table 3 below. In some embodiments, the inert antibody has an Fc isotype listed in Table 3 below.

**Antagonist Antibodies**

A third class of antibodies of the present disclosure includes antagonist antibodies. In some embodiments, antibodies that bind a TREM2 protein may include antagonist antibodies that bind TREM2 and inhibit one or more TREM2 activities, either by preventing interaction between TREM2 and its ligand(s), or by preventing the transduction of signal from the
extracellular domain of TREM2 into the cell cytoplasm in the presence of ligand. In some embodiments, antagonist antibodies of the present disclosure may have the epitope specificity of an agonist antibody of the present disclosure, but have an Fc domain that is not capable of binding Fcγ receptors and thus is unable to, for example, cluster the TREM2 receptor.

[0157] In some embodiments, an antibody of the present disclosure is an antagonist antibody. In some embodiments, the antagonist antibody inhibits one or more TREM2 activities. In some embodiments, the antagonist antibody decreases activity of one or more TREM2-dependent genes. In some embodiments, the one or more TREM2-dependent genes include, without limitation, one or more nuclear factor of activated T-cells (NFAT) transcription factors. In some embodiments, the antagonist antibody decreases the survival of macrophages, microglial cells, M1 macrophages, M1 microglial cells, M2 macrophages, M2 microglial cells, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or dendritic cells. In some embodiments, the antagonist antibody inhibits interaction between TREM2 and one or more TREM2 ligands. In some embodiments, the antagonist antibody inhibits TREM2 signal transduction. In some embodiments, the antagonist antibody inhibits interaction between TREM2 and one or more TREM2 ligands and inhibits TREM2 signal transduction. In some embodiments, the antagonist antibody inhibits TREM2 interaction with DAP12.

[0158] In some embodiments, antibody cross-linking is required for agonist antibody function. Antibody cross-linking can occur through binding to a secondary antibody in vitro or through binding to Fc receptors in vivo. For example, antagonistic antibodies can be converted to agonistic antibodies via biotin/streptavidin cross-linking or secondary antibody binding in vitro (see for example Gravestein et al., (1996) J. Exp. Med. 184:675-685; Gravestein et al., (1994) International Immunol. 7:551-557). Agonistic antibodies may exert their activity by mimicking the biological activity of the receptor ligand or by enhancing receptor aggregation, thereby activating receptor signaling. In some embodiments, the absence of antibody cross-linking is required for antagonistic activity. Antagonistic antibodies may exert their activity by blocking receptor-ligand interactions.
Exemplary antagonist antibody Fc isotypes and modifications are provided in Table 3 below. In some embodiments, the antagonist antibody has an Fc isotype listed in Table 3 below.

Inert and antagonist antibody Fc isotypes

In some embodiments, inert and/or antagonist anti-TREM antibodies have an Fc isotype listed in Table 3 below.

Table 3. Exemplary inert and antagonist anti-TREM2 antibody Fc isotypes

<table>
<thead>
<tr>
<th>Fc Isotype</th>
<th>Mutation (EU numbering scheme)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>N297Aor N297Q</td>
</tr>
<tr>
<td>IgG1</td>
<td>D265A and N297A</td>
</tr>
<tr>
<td>IgG1</td>
<td>L234A and L235A</td>
</tr>
<tr>
<td>IgG2</td>
<td>V234A and G237A</td>
</tr>
<tr>
<td>IgG4</td>
<td>L235A and G237A and E318A and/or F234V E233P and/or F234V N297Aor N297Q</td>
</tr>
<tr>
<td>IgG4</td>
<td>S228P and L236E S241P</td>
</tr>
<tr>
<td>IgG2</td>
<td>H268Q and V309L and A330S and P331S</td>
</tr>
<tr>
<td>IgG1</td>
<td>C220S and C226S and C229S and P238S</td>
</tr>
<tr>
<td>IgG1</td>
<td>C226S and C229S and E233P and L234V, and L235A</td>
</tr>
<tr>
<td>IgG1</td>
<td>E233P and L234V and L235A and G236-deleted P238A</td>
</tr>
<tr>
<td></td>
<td>D265A</td>
</tr>
<tr>
<td></td>
<td>N297A</td>
</tr>
<tr>
<td></td>
<td>A327Q or A327G P329A</td>
</tr>
<tr>
<td>IgG1</td>
<td>K322A and L234A and L235A</td>
</tr>
<tr>
<td>IgG1</td>
<td>L234F and L235E and P331S</td>
</tr>
<tr>
<td>IgG1 or IgG4</td>
<td>T394D</td>
</tr>
<tr>
<td>IgG2</td>
<td>C232S or C233S N297Aor N297Q</td>
</tr>
<tr>
<td>IgG1, IgG2, or IgG4</td>
<td>delta a,b , c, ab, ac, g modifications</td>
</tr>
<tr>
<td>IgG1</td>
<td>Any of the above listed mutations together with A330L or L234F and/or L235E and/or P331S</td>
</tr>
<tr>
<td>IgG1, IgG2, or IgG4</td>
<td>Any of the above listed mutations together with M252Y and/or S254T and/or T256E</td>
</tr>
</tbody>
</table>

In some embodiments, the antibody has an IgG1 isotype with a heavy chain constant region that contains a C220S amino acid substitution according to the EU numbering convention.

In some embodiments, the Fc region further contains one or more additional amino acid substitutions selected from T A330L, L234F; L235E, and/or P331S according to EU numbering convention.

In certain embodiments, the antibody has an IgG2 isotype. In some embodiments, the antibody contains a human IgG2 constant region. In some embodiments, the human IgG2 constant region includes an Fc region. In some embodiments, the Fc region contains one or more modifications. For example, in some embodiments, the Fc region contains one or more amino acid substitutions (e.g., relative to a wild-type Fc region of the same isotype). In some embodiments, the one or more amino acid substitutions are selected from V234A, G237A,
H268E, V309L, N297A, N297Q, A330S, P331S, C232S, C233S, M252Y, S254T, and/or T256E, where the amino acid position is according to the EU numbering convention.

[0165] In certain embodiments, the antibody has an IgG4 isotype. In some embodiments, the antibody contains a human IgG4 constant region. In some embodiments, the human IgG4 constant region includes an Fc region. In some embodiments, the Fc region contains one or more modifications. For example, in some embodiments, the Fc region contains one or more amino acid substitutions (e.g., relative to a wild-type Fc region of the same isotype). In some embodiments, the one or more amino acid substitutions are selected from E233P, F234V, L235A, G237A, E318A (Hutchins et al. (1995) Proc Natl Acad Sci USA, 92:11980-11984), S228P, L236E, S241P, L248E (Reddy et al., (2000) J Immunol, 164:1925-1933; Angal et al., (1993) Mol Immuno, 30(1):105-8; US 8614299 B2), T394D, M252Y, S254T, T256E, and/or N297A, N297Q, where the amino acid position is according to the EU numbering convention.

[0166] In some embodiments, the Fc region further contains one or more additional amino acid substitutions selected from a M252Y, S254T, and/or T256E, where the amino acid position is according to the EU numbering convention.

Further IgG mutations

[0167] In some embodiments, one or more of the IgG1 variants described herein may be combined with an A330L mutation (Lazar et al., (2006) Proc Natl Acad Sci USA, 103:4005-4010), or one or more of L234F, L235E, and/or P331S mutations (Sazinsky et al., (2008) Proc Natl Acad Sci USA, 105:20167-20172), where the amino acid position is according to the EU numbering convention, to eliminate complement activation. In some embodiments, the IgG variants described herein may be combined with one or more mutations to enhance the antibody half-life in human serum (e.g. M252Y, S254T, T256E mutations according to the EU numbering convention) (Dall’Acqua et al., (2006) J Biol Chem, 281:23514-23524; and Strohl e al., (2009) Current Opinion in Biotechnology, 20:685–691).

[0168] In some embodiments, an IgG4 variant of the present disclosure may be combined with an S228P mutation according to the EU numbering convention (Angal et al., (1993) Mol
Immunol, 30:105-108) and/or with one or more mutations described in Peters et al., (2012) J Biol Chem. 13;287(29):24525-33) to enhance antibody stabilization.

Anti-TREM2 antibody characteristics

[0169] In some embodiments, an isolated anti-TREM2 antibody of the present disclosure competes for binding of TREM2 with one or more TREM2 ligands. In some embodiments, the antibody is a human antibody, a humanized antibody, a bispecific antibody, a multivalent antibody, or a chimeric antibody. Exemplary descriptions of such antibodies are found throughout the present disclosure. In some embodiments, the antibody is a bispecific antibody recognizing a first antigen and a second antigen.

[0170] In some embodiments, anti-TREM2 antibodies of the present disclosure bind to a human TREM2, or a homolog thereof, including without limitation a mammalian TREM2 protein, mouse TREM2 protein (Uniprot Accession No. Q99NH8), rat TREM2 protein (Uniprot Accession No. D3ZZ89), Rhesus monkey TREM2 protein (Uniprot Accession No. F6QVF2), bovine TREM2 protein (Uniprot Accession No. Q05B59), equine TREM2 protein (Uniprot Accession No. F7D6L0), pig TREM2 protein (Uniprot Accession No. H2EZZ3), and dog TREM2 protein (Uniprot Accession No. E2RP46). In some embodiments, anti-TREM2 antibodies of the present disclosure specifically bind to human TREM2. In some embodiments, anti-TREM2 antibodies of the present disclosure specifically bind to mouse TREM2. In some embodiments, anti-TREM2 antibodies of the present disclosure specifically bind to both human TREM2 and mouse TREM2. In some embodiments, anti-TREM2 antibodies of the present disclosure modulate (e.g., induce or inhibit) at least one TREM2 activity. In some embodiments, the at least one TREM2 activity includes, without limitation, TREM2 autophosphorylation, DAP12 phosphorylation, Syk phosphorylation; recruitment of Syk, ZAP70, or both to a DAP12/TREM2 complex, PI3K activation, modulated expression of cytokines, modulated expression of pro-inflammatory mediators, ERK phosphorylation, modulated expression of CCR7, modulated of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells, maturation of bone marrow-derived dendritic cells, modulation of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation; modulated ability of
dendritic cells, monocytes, microglia, and/or macrophages to induce T-cell proliferation, modulation of osteoclast production, modulated rate of osteoclastogenesis, or both, modulation of survival and function of microglial cells (such as M1 microglial cells, activated M1 microglial cells, and/or M2 microglial cells), dendritic cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or macrophages (such as M1 macrophages, activated M1 macrophages, and/or M2 macrophages), modulation of one or more types of clearance, modulation of phagocytosis of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, disease-causing proteins, disease-causing peptides, disease-causing nucleic acid (e.g., antisense GGCCCCC (G2C4) repeat-expansion RNA), or tumor cells, modulated secretion of one or more inflammatory cytokines, modulated expression of one or more inflammatory receptors, increased phagocytosis by macrophages, dendritic cells, monocytes, and/or microglial cells under conditions of reduced levels of MCSF, modulated phagocytosis by macrophages, dendritic cells, monocytes, and/or microglial cells in the presence of normal levels of MCSF, modulation of disrupted TREM2/DAP12-dependent gene expression, and/or modulated activity of one or more TREM2-dependent genes.

[0171] In some embodiments, anti-TREM2 antibodies of the present disclosure bind to a TREM2 protein of the present disclosure and/or naturally occurring variants. In certain preferred embodiments, the anti-TREM2 antibodies bind to human TREM2.

[0172] In some embodiments, anti-TREM2 antibodies of the present disclosure are agonist antibodies or antagonist antibodies that bind to a TREM2 protein of the present disclosure expressed on the surface of a cell and modulate (e.g., induce or inhibit) at least one TREM2 activity of the present disclosure after binding to the surface-expressed TREM2 protein. In some embodiments, anti-TREM2 antibodies of the present disclosure are inert antibodies.

[0173] In certain embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 29-112 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 29-112 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 130-171 of human TREM 2 (SEQ ID NO:
1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 130-171 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 139-153 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 139-153 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 139-146 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 139-146 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 130-144 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 130-144 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 158-171 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 158-171 of SEQ ID NO: 1.

[0174] TREM2 proteins of the present disclosure include a complementary determining region 1 (CDR1) located at amino acid residues corresponding to amino acid residues 40-44 of human TREM2 (SEQ ID NO: 1); a complementary determining region 2 (CDR2) located at amino acid residues corresponding to amino acid residues 67-76 of human TREM2 (SEQ ID NO: 1); and a complementary determining region 3 (CDR3) located at amino acid residues corresponding to amino acid residues 114-118 of human TREM2 (SEQ ID NO: 1). Accordingly, in some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 40-44 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 40-44 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 67-76 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 67-76 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 114-118 of human TREM 2 (SEQ ID NO:
1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 114-118 of SEQ ID NO: 1.

[0175] In other embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes amino acid residue Arg47 or Asp87 of human TREM 2 (SEQ ID NO: 1). In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes amino acid residues 40-44 of human TREM 2 (SEQ ID NO: 1). In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes amino acid residues 67-76 of human TREM 2 (SEQ ID NO: 1). In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes amino acid residues 114-118 of human TREM 2 (SEQ ID NO: 1).

[0176] In some embodiments, anti-TREM2 antibodies of the present disclosure competitively inhibit binding of at least one antibody selected from any of the antibodies listed in Table 1. In some embodiments, anti-TREM2 antibodies of the present disclosure competitively inhibit binding of at least one antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87. In some embodiments, anti-TREM2 antibodies of the present disclosure competitively inhibit binding of at least one of the following anti-TREM2 antibodies: Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope of human TREM2 that is the same as or overlaps with the TREM2 epitope bound by at least one antibody selected from any of the antibodies listed in Table 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope of human TREM2 that is the same as or overlaps with the TREM2 epitope bound by at least one antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37.
Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab52, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope of human TREM2 that is the same as or overlaps with the TREM2 epitope bound by at least one of the following anti-TREM2 antibodies: Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65. In some embodiments, anti-TREM2 antibodies of the present disclosure bind essentially the same TREM2 epitope bound by at least one antibody selected from any of the antibodies listed in Table 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind essentially the same TREM2 epitope bound by at least one antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87. In some embodiments, anti-TREM2 antibodies of the present disclosure bind essentially the same TREM2 epitope bound by at least one of the following anti-TREM2 antibodies: Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) “Epitope Mapping Protocols,” in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ).

[0177] In an exemplary competition assay, immobilized TREM2 or cells expressing TEM2 on the cell surface are incubated in a solution comprising a first labeled antibody that binds to TREM2 (e.g., human or non-human primate) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to TREM2. The second antibody may be present in a hybridoma supernatant. As a control, immobilized TREM2 or cells expressing TREM2 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to TREM2, excess unbound antibody is removed, and the amount of label associated
with immobilized TREM2 or cells expressing TREM2 is measured. If the amount of label associated with immobilized TREM2 or cells expressing TREM2 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to TREM2. See, Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[0178] In some embodiments, anti-TREM2 antibodies of the present disclosure comprise (a) a heavy chain variable region comprising at least one, two, or three HVRs selected from HVR-H1, HVR-H2, and HVR-H3 of any one of the antibodies listed in Table 1 or selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87; and/or (b) a light chain variable region comprising at least one, two, or three HVRs selected from HVR-L1, HVR-L2, and HVR-L3 of any one of the antibodies listed in Table 1 or selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87. In some embodiments, the HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 comprise Kabat CDR, Chothia CDR, or Contact CDR sequences as shown in Table 1 or from an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56,
Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87.

In some embodiments, anti-TREM2 antibodies of the present disclosure comprise at least one, two, three, four, five, or six HVRs selected from (i) HVR-H1 comprising the amino acid sequence of any of the HVR-H1 sequences listed in Table 1 or from an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87; (ii) HVR-H2 comprising the amino acid sequence of any of the HVR-H2 sequences listed in Table 1 or from an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87; (iii) HVR-H3 comprising the amino acid sequence of any of the HVR-H3 sequences listed in Table 1 or from an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87; (iv) HVR-L1 comprising the amino acid sequence of any of the HVR-L1 sequences listed in Table 1 or from an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11,
Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87; (v) HVR-L2 comprising the amino acid sequence of any of the HVR-L2 sequences listed in Table 1 or from an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87; and (vi) HVR-L3 comprising the amino acid sequence of any of the HVR-L3 sequences listed in Table 1 or from an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87.

[0180] In some embodiments, anti-TREM2 antibodies of the present disclosure comprise a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises one or more of: (a) an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24; (b) an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49; and (c) an HVR-H3
comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50-119, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 50-119; and/or wherein the light chain variable domain comprises one or more of: (a) an HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 120-137, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 120-137; (b) an HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152; and (c) an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 153-236 or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152.

[0181] In some embodiments, anti-TREM2 antibodies of the present disclosure comprise a heavy chain variable region of any one of the antibodies listed in Table 1 or selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab52, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87; and/or a light chain variable region of any one of the antibodies listed in Table 1 or selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab52, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87.

[0182] Any of the antibodies of the present disclosure may be produced by a cell line. In some embodiments, the cell line may be a yeast cell line. In other embodiments, the cell line
may be a mammalian cell line. In certain embodiments, the cell line may be a hybridoma cell line. Any cell line known in the art suitable for antibody production may be used to produce an antibody of the present disclosure. Exemplary cell lines for antibody production are described throughout the present disclosure.

[0183] In some embodiments, the anti-TREM2 antibody is an anti-TREM2 monoclonal antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.

[0184] In some embodiments, the anti-TREM2 antibody is anti-TREM2 monoclonal antibody Ab1. In some embodiments, the anti-TREM2 antibody is an isolated antibody which binds essentially the same TREM2 epitope as Ab1. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody Ab1. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab1. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains and the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab1. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.

[0185] In some embodiments, the anti-TREM2 antibody is anti-TREM2 monoclonal antibody Ab9. In some embodiments, the anti-TREM2 antibody is an isolated antibody which binds essentially the same TREM2 epitope as Ab9. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy
chain variable domains of monoclonal antibody Ab9. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab9. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains and the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab9. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.

[0186] In some embodiments, the anti-TREM2 antibody is anti-TREM2 monoclonal antibody Ab14. In some embodiments, the anti-TREM2 antibody is an isolated antibody which binds essentially the same TREM2 epitope as Ab14. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody Ab14. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab14. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains and the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab14. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.

[0187] In some embodiments, the anti-TREM2 antibody is anti-TREM2 monoclonal antibody Ab22. In some embodiments, the anti-TREM2 antibody is an isolated antibody which binds essentially the same TREM2 epitope as Ab22. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody Ab22. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab22. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains and the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab22. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.
[0188] In some embodiments, the anti-TREM2 antibody is anti-TREM2 monoclonal antibody Ab45. In some embodiments, the anti-TREM2 antibody is an isolated antibody which binds essentially the same TREM2 epitope as Ab45. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody Ab45. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab45. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.

[0189] In some embodiments, the anti-TREM2 antibody is anti-TREM2 monoclonal antibody Ab65. In some embodiments, the anti-TREM2 antibody is an isolated antibody which binds essentially the same TREM2 epitope as Ab65. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody Ab65. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab65. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.

[0190] In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 43-50 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 43-50 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to one or more amino acids within amino acid residues 49-57 of human TREM 2 (SEQ ID NO: 1), or within amino acid residues on a TREM2 protein corresponding to amino acid residues 49-57 of SEQ ID NO: 1. In some embodiments, anti-TREM2 antibodies of the present disclosure bind
to an epitope that includes one or more amino acid residues within amino acid residues 43-50 of human TREM 2 (SEQ ID NO: 1). In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope that includes one or more amino acid residues within amino acid residues 49-57 of human TREM 2 (SEQ ID NO: 1). In some embodiments, anti-TREM2 antibodies of the present disclosure competitively inhibit binding of at least one of the following anti-TREM2 antibodies: Ab21 and Ab52. In some embodiments, anti-TREM2 antibodies of the present disclosure bind to an epitope of human TREM2 that is the same as or overlaps with the TREM2 epitope bound by at least one of the following anti-TREM2 antibodies: Ab21 and Ab52. In some embodiments, anti-TREM2 antibodies of the present disclosure comprise (a) a heavy chain variable region comprising at least one, two, or three HVRs selected from HVR-H1, HVR-H2, and HVR-H3 of any one of antibodies Ab21 and Ab52; and/or (b) a light chain variable region comprising at least one, two, or three HVRs selected from HVR-L1, HVR-L2, and HVR-L3 of any one of antibodies Ab21 and Ab52. In some embodiments, the HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 comprise Kabat CDR, Chothia CDR, or Contact CDR sequences as shown in Table 1. In some embodiments, anti-TREM2 antibodies of the present disclosure comprise a heavy chain variable region of any one of antibodies Ab21 and Ab52; and/or a light chain variable region of any one of antibodies Ab21 and Ab52. In some embodiments, the anti-TREM2 antibody is anti-TREM2 monoclonal antibody Ab52 or Ab21. In some embodiments, the anti-TREM2 antibody is an isolated antibody which binds essentially the same TREM2 epitope as Ab52 or Ab21. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.

[0191] In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody Ab52 or Ab21. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab52 or Ab21. In some embodiments, the anti-TREM2 antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains and the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody Ab52 or Ab21. In some embodiments, anti-TREM2 antibodies of the
present disclosure comprise at least one, two, three, four, five, or six HVRs selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:398, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:398; (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 399, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:399; (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:400, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:400; (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:401 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:401; (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:402 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:402; and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:403, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:403. In some embodiments, anti-TREM2 antibodies of the present disclosure comprise at least one, two, three, four, five, or six HVRs selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:404, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:404; (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 405, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:405; (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:406, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:406; (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:407, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:407; (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:408, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:408; and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:409, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:409.

[0192] In some embodiments, anti-TREM2 antibodies of the present disclosure comprise a heavy chain variable region, and the heavy chain variable region comprises at least one, at least two, or three HVR sequences selected from those listed in Table 1; and/or comprise a light chain
variable region, and the light chain variable region comprises at least one, at least two, or three HVR sequences selected from those listed in Table 1.

In some embodiments, anti-TREM2 antibodies of the present disclosure compete for binding of TREM2 with one or more TREM2 ligands. Examples of suitable TREM2 ligands include, without limitation, TREM2 ligands expressed by *E. coli* cells, apoptotic cells, nucleic acids, anionic lipids, zwitterionic lipids, negatively charged phospholipids, phosphatidylserine, sulfatides, phosphatidylcholines, sphingomyelins, membrane phospholipids, lipidated proteins, proteolipids, lipidated peptides, and lipidated amyloid beta peptide. Accordingly, in certain embodiments, the one or more TREM2 ligands comprise *E. coli* cells, apoptotic cells, nucleic acids, anionic lipids, zwitterionic lipids, negatively charged phospholipids, phosphatidylserine, sulfatides, phosphatidylcholines, sphingomyelins, phospholipids, lipidated proteins, proteolipids, lipidated peptides, and lipidated amyloid beta peptide. In some embodiments, the anti-TREM2 antibody is an agonist antibody. In some embodiments, the anti-TREM2 antibody is an inert antibody. In some embodiments, the anti-TREM2 antibody is an antagonist antibody.

The dissociation constants (K_{d}) of anti-TREM2 antibodies for human TREM2 (e.g., human TREM2-Fc fusion proteins and human monomeric TREM2 proteins) and mouse TREM2 (e.g., mouse TREM2-Fc fusion proteins) may be less than 10 nM, less than 9.5 nM, less than 9 nM, less than 8.5 nM, less than 8 nM, less than 7.5 nM, less than 7 nM, less than 6.9 nM, less than 6.8 nM, less than 6.7 nM, less than 6.6 nM, less than 6.5 nM, less than 6.4 nM, less than 6.3 nM, less than 6.2 nM, less than 6.1 nM, less than 6 nM, less than 5.5 nM, less than 5 nM, less than 4.5 nM, less than 4 nM, less than 3.5 nM, less than 3 nM, less than 2.5 nM, less than 2 nM, less than 1.5 nM, less than 1 nM, less than 0.95 nM, less than 0.9 nM, less than 0.85 nM, less than 0.8 nM, less than 0.75 nM, less than 0.7 nM, less than 0.65 nM, less than 0.6 nM, less than 0.55 nM, less than 0.5 nM, less than 0.45 nM, less than 0.4 nM, less than 0.35 nM, less than 0.3 nM, less than 0.29 nM, less than 0.28 nM, less than 0.27 nM, less than 0.26 nM, less than 0.25 nM, less than 0.24 nM, less than 0.23 nM, less than 0.22 nM, less than 0.21 nM, less than 0.2 nM, less than 0.15 nM, or less than 0.1 nM. In some embodiments, dissociation constants range from less than about 6.70 nM to less than about 0.23 nM. In some embodiments, dissociation constants of anti-TREM2 antibodies for human TREM2-Fc fusion proteins range from less than
about 0.71 nM to less than about 0.23 nM. In some embodiments, dissociation constants of anti-TREM2 antibodies for human monomeric TREM2 proteins range from less than about 6.70 nM to less than about 0.66 nM. In some embodiments, dissociation constants of anti-TREM2 antibodies for mouse TREM2-Fc fusion proteins range from less than about 4.90 nM to less than about 0.35 nM. Dissociation constants may be determined through any analytical technique, including any biochemical or biophysical technique such as ELISA, surface plasmon resonance (SPR), bio-layer interferometry (see, e.g., Octet System by ForteBio), isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), circular dichroism (CD), stopped-flow analysis, and colorimetric or fluorescent protein melting analyses. In certain embodiments, the anti-TREM2 antibody is an agonist antibody. In other embodiments, the anti-TREM2 antibody is an antagonist antibody.

[0195] Additional anti-TREM2 antibodies, e.g., antibodies that specifically bind to a TREM2 protein of the present disclosure, may be identified, screened, and/or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

**Bispecific antibodies**

[0196] Certain aspects of the present disclosure relate to bispecific antibodies that bind to a TREM2 protein of the present disclosure and a second antigen. Methods of generating bispecific antibodies are well known in the art and described herein. In some embodiments, bispecific antibodies of the present disclosure bind to one or more amino acid residues of human TREM2 (SEQ ID NO: 1), or amino acid residues on a TREM2 protein corresponding to amino acid residues of SEQ ID NO: 1. In other embodiments, bispecific antibodies of the present disclosure also bind to one or more amino acid residues of human DAP12 (SEQ ID NO: 2), or amino acid residues on a DAP12 protein corresponding to amino acid residues of SEQ ID NO: 2.

[0197] In some embodiments, bispecific antibodies of the present disclosure recognize a first antigen and a second antigen. In some embodiments, the first antigen is human TREM2 or a naturally occurring variant thereof, or human DAP12 or a naturally occurring variant thereof. In some embodiments, the second antigen is a disease-causing protein selected from amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc,
huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein A1, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, and proline-arginine (PR) repeat peptides. In some embodiments, the second antigen is a blood brain barrier targeting protein selected from transferrin receptor, insulin receptor, insulin like growth factor receptor, LRP-1, and LRP1; or ligands and/or proteins expressed on immune cells, wherein the ligands and/or proteins selected from the group consisting of: CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, BTLA, KIR, GAL.9, TIM3, A2AR, LAG, and phosphatidylinerine. Alternatively, the second antigen may be, without limitation, a protein expressed on one or more tumor cells.

Antibody fragments

[0198] Certain aspects of the present disclosure relate to antibody fragments that bind to one or more of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of human TREM2. In some embodiments, the antibody fragment is an Fab, Fab’, Fab’-SH, F(ab’)_2, Fv or scFv fragment. In some embodiments, the antibody fragment is used in combination with one or more antibodies that specifically bind a disease-causing protein selected from: amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein A1, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, proline-arginine (PR) repeat peptides, and any combination thereof, and/or one or more antibodies that specifically bind a cancer-associated protein selected from: CD40, OX40, ICOS,
CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, BTLA, KIR, GAL9, TIM3, A2AR, LAG, phosphatidylserine, and any combination thereof.

Antibody frameworks

[0199] Any of the antibodies described herein further include a framework. In some embodiments, the framework is a human immunoglobulin framework. For example, in some embodiments, an antibody (e.g., an anti-TREM2 antibody) comprises HVRs as in any of the above embodiments and further comprises an acceptor human framework, e.g., a human immunoglobulin framework or a human consensus framework. Human immunoglobulin frameworks may be part of the human antibody, or a non-human antibody may be humanized by replacing one or more endogenous frameworks with human framework region(s). Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. J. Immunol. 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. Proc. Natl. Acad. Sci. USA, 89:4285 (1992); and Presta et al. J. Immunol., 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., J. Biol. Chem. 272:10678-10684 (1997) and Rosok et al., J. Biol. Chem. 271:22611-22618 (1996)).

[0200] In some embodiments, an antibody comprises a heavy chain variable region comprising an HVR-H1, an HVR-H2, and an HVR-H3 of the present disclosure and one, two, three or four of the heavy chain framework regions as shown in FIG. 2B. In some embodiments, an antibody comprises a light chain variable region comprising an HVR-L1, an HVR-L2, and an HVR-L3 of the present disclosure and one, two, three or four of the light chain framework regions as shown in FIG. 2C. In some embodiments, an antibody comprises a heavy chain variable region comprising an HVR-H1, an HVR-H2, and an HVR-H3 of the present disclosure and one, two, three or four of the heavy chain framework regions as shown in FIG. 2B and further comprises a light chain variable region comprising an HVR-L1, an HVR-L2, and an
HVR-L3 of the present disclosure and one, two, three or four of the light chain framework regions as shown in FIG. 2C.

**PI3K activation**

[0201] In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce PI3K activation after binding to a TREM2 protein expressed in a cell.

[0202] PI3Ks are a family of related intracellular signal transducer kinases capable of phosphorylating the 3-position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns). The PI3K family is divided into three different classes (Class I, Class II, and Class III) based on primary structure, regulation, and in vitro lipid substrate specificity.

[0203] Activated PI3K produces various 3-phosphorylated phosphoinositides, including without limitation, PtdIns3P, PtdIns(3,4)P2, PtdIns(3,5)P2, and PtdIns(3,4,5)P3. These 3-phosphorylated phosphoinositides function in a mechanism by which signaling proteins are recruited to various cellular membranes. These signaling proteins contain phosphoinositide-binding domains, including without limitation, PX domains, pleckstrin homology domains (PH domains), and FYVE domains. Any method known in the art for determining PI3K activation may be used.

[0204] Without wishing to be bound by theory, it is believed that anti-TREM2 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of PI3K activity, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Tauopathy disease, and/or multiple sclerosis.

**Increased expression of cytokines**

[0205] In some embodiments, the anti-TREM2 antibodies of the present disclosure have an anti-inflammatory activity in the brain after binding to a TREM2 protein expressed on a cell surface. It has recently been reported that TREM2 has an anti-inflammatory role in the brain. In certain embodiments, the anti-TREM2 antibodies of the present disclosure increase the
expression of cytokines (e.g., anti-inflammatory mediators) and/or reduce the expression of pro-inflammatory mediators after binding to a TREM2 protein expressed in a cell.

[0206] Inflammation is part of a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, and irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by an organism to remove the injurious stimuli and to initiate the healing process. Inflammation can be classified as either acute inflammation or chronic inflammation. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Chronic inflammation is prolonged inflammation that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

[0207] As used herein, anti-inflammatory mediators are proteins involved either directly or indirectly (e.g., by way of an anti-inflammatory signaling pathway) in a mechanism that reduces, inhibits, or inactivates an inflammatory response. Any method known in the art for identifying and characterizing anti-inflammatory mediators may be used. Examples of anti-inflammatory mediators include, without limitation, cytokines, such as IL-12p70, IL-6, and IL-10.

[0208] In some embodiments, the anti-TREM2 antibodies of the present disclosure may increase expression of cytokines, such as IL-12p70, IL-6, and IL-10. In certain embodiments, increased expression of the cytokines occurs in macrophages, dendritic cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglial cells. Increased expression may include, without limitation, in increase in gene expression, an increase in transcriptional expression, or an increase in protein expression. Any method known in the art for determining gene, transcript (e.g., mRNA), and/or protein expression may be used. For example, Northern blot analysis may be used to determine cytokine gene expression levels, RT-PCR may be used to
determine the level of cytokine transcription, and Western blot analysis may be used to determine cytokine protein levels.

[0209] As used herein, a cytokine may have increased expression if its expression in one or more cells of a subject treated with an anti-TREM2 antibody of the present disclosure is greater than the expression of the same cytokine expressed in one or more cells of a corresponding subject that is not treated with the anti-TREM2 antibody. In some embodiments, an anti-TREM2 antibody of the present disclosure may increase cytokine expression in one or more cells of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to cytokine expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 antibody. In other embodiments, an anti-TREM2 antibody of the present disclosure increases cytokine expression in one or more cells of a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to cytokine expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 antibody.

[0210] Without wishing to be bound by theory, it is believed that, in some embodiments, anti-TREM2 antibodies of the present disclosure are useful for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of one or more anti-inflammatory mediators, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, and/or multiple sclerosis.
Reduced expression of pro-inflammatory mediators

[0211] In some embodiments, the anti-TREM2 antibodies of the present disclosure may decrease the expression of pro-inflammatory mediators after binding to a TREM2 protein expressed in a cell.

[0212] As used herein, pro-inflammatory mediators are proteins involved either directly or indirectly (e.g., by way of pro-inflammatory signaling pathways) in a mechanism that induces, activates, promotes, or otherwise increases an inflammatory response. Any method known in the art for identifying and characterizing pro-inflammatory mediators may be used. Examples of pro-inflammatory mediators include, without limitation, cytokines such as IFN-a4, IFN-b, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, and CRP.

[0213] In some embodiments, the anti-TREM2 antibodies of the present disclosure may decrease functional expression and/or secretion of pro-inflammatory mediators, such as IFN-a4, IFN-b, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, and CRP. In certain embodiments, decreased expression of the pro-inflammatory mediators occurs in macrophages, dendritic cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglial cells. Decreased expression may include, without limitation, a decrease in gene expression, a decrease in transcriptional expression, or a decrease in protein expression. Any method known in the art for determining gene, transcript (e.g., mRNA), and/or protein expression may be used. For example, Northern blot analysis may be used to determine pro-inflammatory mediator gene expression levels, RT-PCR may be used to determine the level of pro-inflammatory mediator transcription, and Western blot analysis may be used to determine pro-inflammatory mediator protein levels.

[0214] In certain embodiments, pro-inflammatory mediators include inflammatory cytokines. Accordingly, in certain embodiments, the anti-TREM2 antibodies of the present disclosure may reduce secretion of one or more inflammatory cytokines. Examples of inflammatory cytokines
whose secretion may be reduced by the anti-TREM2 antibodies of the present disclosure include, without limitation, IFN-α, IFN-β, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, CRP, and MCP-1.

[0215] In certain embodiments, pro-inflammatory mediators include inflammatory receptors. Accordingly, in certain embodiments, the anti-TREM2 antibodies of the present disclosure may reduce expression of one or more inflammatory receptors. Examples of inflammatory receptors whose expression may be reduced by the anti-TREM2 antibodies of the present disclosure include, without limitation, CD86.

[0216] As used herein, a pro-inflammatory mediator may have decreased expression if its expression in one or more cells of a subject treated with an agonist anti-TREM2 antibody of the present disclosure is less than the expression of the same pro-inflammatory mediator expressed in one or more cells of a corresponding subject that is not treated with the agonist anti-TREM2 antibody. In some embodiments, the agonist anti-TREM2 antibody of the present disclosure may decrease pro-inflammatory mediator expression in one or more cells of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to pro-inflammatory mediator expression in one or more cells of a corresponding subject that is not treated with the agonist anti-TREM2 antibody. In other embodiments, the agonist anti-TREM2 antibody may decrease pro-inflammatory mediator expression in one or more cells of a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to pro-
inflammatory mediator expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 antibody.

[0217] Without wishing to be bound by theory, it is believed that some anti-TREM2 antibodies of the present disclosure may be useful for preventing, lowering the risk of, or treating conditions and/or diseases associated with increased levels of one or more pro-inflammatory mediators, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, and/or multiple sclerosis.

*ERK phosphorylation*

[0218] In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce extracellular signal-regulated kinase (ERK) phosphorylation after binding to a TREM2 protein expressed in a cell.

[0219] Extracellular signal-regulated kinases (ERKs) are widely expressed protein kinase intracellular signaling kinases that are involved in, for example, the regulation of meiosis, mitosis, and postmitotic functions in differentiated cells. Various stimuli, such as growth factors, cytokines, virus infection, ligands for heterotrimeric G protein-coupled receptors, transforming agents, and carcinogens, activate ERK pathways. Phosphorylation of ERKs leads to the activation of their kinase activity.

[0220] Without wishing to be bound by theory, it is believed that anti-TREM2 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of ERK phosphorylation, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, and/or multiple sclerosis.

*Syk phosphorylation*

[0221] In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce spleen tyrosine kinase (Syk) phosphorylation after binding to a TREM2 protein expressed in a cell.
Spleen tyrosine kinase (Syk) is an intracellular signaling molecule that functions downstream of TREM2 by phosphorylating several substrates, thereby facilitating the formation of a signaling complex leading to cellular activation and inflammatory processes.

Without wishing to be bound by theory, it is believed that anti-TREM2 and/or anti-DAP12 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of Syk phosphorylation, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, and/or multiple sclerosis.

**TREM2 autophosphorylation**

In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce TREM2 autophosphorylation after binding to a TREM2 protein expressed in a cell.

Without wishing to be bound by theory, it is believed that anti-TREM2 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of TREM2 phosphorylation, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, and/or multiple sclerosis.

**DAP12 binding and phosphorylation**

In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce binding of TREM2 to DAP12. In other embodiments, the anti-TREM2 antibodies of the present disclosure may induce DAP12 phosphorylation after binding to a TREM2 protein expressed in a cell. In other embodiments, TREM2-mediated DAP12 phosphorylation is induced by one or more SRC family tyrosine kinases. Examples of SRC family tyrosine kinases include, without limitation, Src, Syk, Yes, Fyn, Fgr, Lck, Hck, Blk, Lyn, and Frk.

DAP12 is variously referred to as TYRO protein tyrosine kinase-binding protein, TYROBP, KARAP, and PLOS. DAP12 is a transmembrane signaling protein that contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. In certain
embodiments, the anti-TREM2 and/or anti-DAP12 antibody may induce DAP12 phosphorylation in its ITAM motif. Any method known in the art for determining protein phosphorylation, such as DAP12 phosphorylation, may be used.

[0228] In some embodiments, DAP12 is phosphorylated by SRC family kinases, resulting in the recruitment and activation of the Syk kinase, ZAP70 kinase, or both, to a DAP12/TREM2 complex. Thus, in certain embodiments, the anti-TREM2 antibodies of the present disclosure may recruit Syk, ZAP70, or both to a DAP12/TREM2 complex.

[0229] Without wishing to be bound by theory, it is believed that anti-TREM2 antibodies of the present disclosure are useful for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of DAP12 activity, DAP12 phosphorylation, or recruitment of Syk, ZAP70, or both to a DAP12/TREM2 complex, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Tauopathy disease, and/or multiple sclerosis.

*Increased expression of C-C chemokine receptor 7*

[0230] In some embodiments, the anti-TREM2 antibodies of the present disclosure may increase expression of C-C chemokine receptor 7 (CCR7) after binding to a TREM2 protein expressed in a cell. Increased expression may include, without limitation, in increase in gene expression, an increase in transcriptional expression, or an increase in protein expression. Any method known in the art for determining gene, transcript (*e.g.*, mRNA), and/or protein expression may be used. For example, Northern blot analysis may be used to determine anti-inflammatory mediator gene expression levels, RT-PCR may be used to determine the level of anti-inflammatory mediator transcription, and Western blot analysis may be used to determine anti-inflammatory mediator protein levels.

[0231] C-C chemokine receptor 7 (CCR7) is a member of the G protein-coupled receptor family. CCR7 is expressed in various lymphoid tissues and can activate B-cells and T-cells. In some embodiments, CCR7 may modulate the migration of memory T-cells to secondary lymphoid organs, such as lymph nodes. In other embodiments, CCR7 may stimulate dendritic
cell maturation. CCR7 is a receptor protein that can bind the chemokine (C-C motif) ligands CCL19/ELC and CCL21.

[0232] As used herein, CCR7 may have increased expression if its expression in one or more cells of a subject treated with an anti-TREM2 antibody of the present disclosure is greater than the expression of CCR7 expressed in one or more cells of a corresponding subject that is not treated with the anti-TREM2 antibody. In some embodiments, an anti-TREM2 antibody of the present disclosure may increase CCR7 expression in one or more cells of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to CCR7 expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 antibody. In other embodiments, an anti-TREM2 antibody of the present disclosure increases CCR7 expression in one or more cells of a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to CCR7 expression in one or more cells of a corresponding subject that is not treated with the anti-TREM2 antibody.

[0233] In some embodiments, increased expression of CCR7 occurs in macrophages, dendritic cells, and/or microglial cells. Increased expression of CCR7 may induce microglial cell chemotaxis toward cells expressing the chemokines CCL19 and CCL21. Accordingly, in certain embodiments, anti-TREM2 antibodies of the present disclosure may induce microglial cell chemotaxis toward CCL19 and CCL21 expressing cells.
Without wishing to be bound by theory, it is believed that, in some embodiments, anti-TREM2 antibodies of the present disclosure are useful for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of CCR7, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease.

Enhancement or normalization of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation

In some embodiments, the anti-TREM2 antibodies of the present disclosure may enhance and/or normalize the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation after binding to a TREM2 protein expressed in a cell.

In some embodiments, agonist anti-TREM2 antibodies of the present disclosure may enhance and/or normalize the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation in one or more bone marrow-derived dendritic cells of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation in one or more bone marrow-derived dendritic cells of a corresponding subject that is not treated with the agonist anti-TREM2 antibody. In other embodiments, the agonist anti-TREM2 antibody may enhance and/or normalize the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation in one or more bone marrow-derived dendritic cells of a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least
7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation in one or more bone marrow-derived dendritic cells of a corresponding subject that is not treated with the agonist anti-TREM2 antibody.

[0237] Without wishing to be bound by theory, it is believed that anti-TREM2 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with a decreased or deregulated ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, and/or multiple sclerosis.

Osteoclast production

[0238] In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce osteoclast production and/or increase the rate of osteoclastogenesis after binding to a TREM2 protein expressed in a cell.

[0239] As used herein, an osteoclast is a type of bone cell that can remove bone tissue by removing its mineralized matrix and breaking up the organic bone (e.g., bone resorption). Osteoclasts can be formed by the fusion of cells of the monocyte-macrophage cell line. In some embodiments, osteoclasts may be characterized by high expression of tartrate resistant acid phosphatase (TRAP) and cathepsin K.

[0240] As used herein, the rate of osteoclastogenesis may be increased if the rate of osteoclastogenesis in a subject treated with an agonist anti-TREM2 antibody of the present disclosure is greater than the rate of osteoclastogenesis in a corresponding subject that is not treated with the agonist anti-TREM2 antibody. In some embodiments, an agonist anti-TREM2 antibody of the present disclosure may increase the rate of osteoclastogenesis in a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least
150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to rate of osteoclastogenesis in a corresponding subject that is not treated with the agonist anti-TREM2 antibody. In other embodiments, an agonist anti-TREM2 antibody of the present disclosure may increase the rate of osteoclastogenesis in a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to rate of osteoclastogenesis in a corresponding subject that is not treated with the agonist anti-TREM2 antibody.

[0241] As used herein, the rate of osteoclastogenesis may be decreased if the rate of osteoclastogenesis in a subject treated with an antagonist anti-TREM2 antibody of the present disclosure is smaller than the rate of osteoclastogenesis in a corresponding subject that is not treated with the antagonist anti-TREM2 antibody. In some embodiments, an antagonist anti-TREM2 antibody of the present disclosure may decrease the rate of osteoclastogenesis in a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 160%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to rate of osteoclastogenesis in a corresponding subject that is not treated with the antagonist anti-TREM2 antibody. In other embodiments, an antagonist anti-TREM2 antibody of the present disclosure may decrease the rate of osteoclastogenesis in a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold.
fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to rate of osteoclastogenesis in a corresponding subject that is not treated with the antagonist anti-TREM2 antibody.

[0242] Without wishing to be bound by theory, it is believed that anti-TREM2 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with abnormal bone formation and maintenance including osteoporosis, which is associated with pathological decrease in bone density and osteoporotic diseases which are associated with pathological increase in bone density.

Proliferation and survival of TREM2-expressing cells

[0243] In some embodiments, the anti-TREM2 antibodies of the present disclosure may increase the proliferation, survival, and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and microglial cells (microglia) after binding to TREM2 protein expressed in a cell.

[0244] Microglial cells are a type of glial cell that are the resident macrophages of the brain and spinal cord, and thus act as the first and main form of active immune defense in the central nervous system (CNS). Microglial cells constitute 20% of the total glial cell population within the brain. Microglial cells are constantly scavenging the CNS for plaques, damaged neurons and infectious agents. The brain and spinal cord are considered "immune privileged" organs in that they are separated from the rest of the body by a series of endothelial cells known as the blood–brain barrier, which prevents most infections from reaching the vulnerable nervous tissue. In the case where infectious agents are directly introduced to the brain or cross the blood–brain barrier, microglial cells must react quickly to decrease inflammation and destroy the infectious agents before they damage the sensitive neural tissue. Due to the unavailability of antibodies from the rest of the body (few antibodies are small enough to cross the blood brain barrier), microglia must be able to recognize foreign bodies, swallow them, and act as antigen-presenting cells activating T-cells. Since this process must be done quickly to prevent potentially fatal damage, microglial cells are extremely sensitive to even small pathological changes in the CNS. They
achieve this sensitivity in part by having unique potassium channels that respond to even small changes in extracellular potassium.

[0245] As used herein, macrophages of the present disclosure include, without limitation, M1 macrophages, activated M1 macrophages, and M2 macrophages. As used herein, microglial cells of the present disclosure include, without limitation, M1 microglial cells, activated M1 microglial cells, and M2 microglial cells.

[0246] In some embodiments, anti-TREM2 antibodies of the present disclosure may increase the expression of CD83 and/or CD86 on dendritic cells, monocytes, and/or macrophages.

[0247] As used herein, the rate of proliferation, survival, and/or function of macrophages, dendritic cells, monocytes, and/or microglia may include increased expression if the rate of proliferation, survival, and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia in a subject treated with an anti-TREM2 antibody of the present disclosure is greater than the rate of proliferation, survival, and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia in a corresponding subject that is not treated with the anti-TREM2 antibody. In some embodiments, an anti-TREM2 antibody of the present disclosure may increase the rate of proliferation, survival, and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia in a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 158%, at least 170%, at least 180%, at least 190%, or at least 200% for example, as compared to the rate of proliferation, survival, and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia in a corresponding subject that is not treated with the anti-TREM2 antibody. In other embodiments, an anti-TREM2 antibody of the present disclosure may increase the rate of proliferation, survival, and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin,
Kupffer cells, and/or microglia in a subject by at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.15 fold, at least 2.2 fold, at least 2.25 fold, at least 2.3 fold, at least 2.35 fold, at least 2.4 fold, at least 2.45 fold, at least 2.5 fold, at least 2.55 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10 fold, for example, as compared to the rate of proliferation, survival, and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia in a corresponding subject that is not treated with the anti-TREM2 antibody.

[0248] Without wishing to be bound by theory, it is believed that anti-TREM2 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with a reduction in proliferation, survival, and/or function of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia including d dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Taopathy disease.

**Clearance and phagocytosis**

[0249] In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce clearance and/or phagocytosis after binding to a TREM2 protein expressed in a cell of one or more of apoptotic neurons, nerve tissue debris of the nervous system, non-nerve tissue debris of the nervous system, bacteria, other foreign bodies, disease-causing proteins, disease-causing peptides, disease-causing nucleic acid, or tumor cells. In certain embodiments, disease-causing proteins include, without limitation, amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein A1, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, and Repeat-associated non-ATG (RAN) translation products including DiPeptide
Repeats, (DPRs peptides) composed of glycine-alanine (GA), glycine-proline (GP), glycine-arginine (GR), proline-alanine (PA), or proline-arginine (PR). In certain embodiments, disease-causing nucleic acids include, without limitation, antisense GGCCCC (G2C4) repeat-expansion RNA.

[0250] In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce one or more types of clearance, including without limitation, apoptotic neuron clearance, nerve tissue debris clearance, non-nerve tissue debris clearance, bacteria or other foreign body clearance, disease-causing protein clearance, disease-causing peptide clearance, disease-causing nucleic acid clearance, and tumor cell clearance.

[0251] In some embodiments, the anti-TREM2 antibodies of the present disclosure may induce phagocytosis of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, disease-causing proteins, disease-causing peptides, disease-causing nucleic acid, and/or tumor cells.

[0252] In some embodiments, the anti-TREM2 antibodies of the present disclosure may increase phagocytosis by macrophages, dendritic cells, monocytes, and/or microglia under conditions of reduced levels of macrophage colony-stimulating factor (MCSF). Alternatively, in some embodiments, the anti-TREM2 antibodies of the present disclosure may decrease phagocytosis by macrophages, dendritic cells, monocytes, and/or microglia in the presence of normal levels of macrophage colony-stimulating factor (MCSF).

[0253] Without wishing to be bound by theory, it is believed that anti-TREM2 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with apoptotic neurons, nerve tissue debris of the nervous system, non-nerve tissue debris of the nervous system, bacteria, other foreign bodies, or disease-causing proteins, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, Parkinson’s disease, Amyotrophic lateral sclerosis, Huntington’s disease, Tauopathy disease, and/or multiple sclerosis.
TREM2-dependent gene expression

[0254] In some embodiments, agonist anti-TREM2 antibodies of the present disclosure may increase the activity and/or expression of TREM2-dependent genes, such as one or more transcription factors of the nuclear factor of activated T-cells (NFAT) family of transcription factors. Alternatively, antagonistic anti-TREM2 antibodies of the present disclosure may inhibit the activity and/or expression of TREM2-dependent genes, such as one or more transcription factors of the NFAT family of transcription factors.

[0255] Without wishing to be bound by theory, it is believed that agonist anti-TREM2 antibodies of the present disclosure are beneficial for preventing, lowering the risk of, or treating conditions and/or diseases associated with decreased levels of TREM2-dependent genes, including dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and/or multiple sclerosis.

Antibody preparation

[0256] Anti-TREM2 antibodies of the present disclosure can encompass polyclonal antibodies, monoclonal antibodies, humanized and chimeric antibodies, human antibodies, antibody fragments (e.g., Fab, Fab’-SH, Fv, scFv, and F(ab’)2), bispecific and polyspecific antibodies, multivalent antibodies, library derived antibodies, antibodies having modified effector functions, fusion proteins containing an antibody portion, and any other modified configuration of the immunoglobulin molecule that includes an antigen recognition site, such as an epitope having amino acid residues of a TREM2 protein of the present disclosure, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The anti-TREM2 antibodies may be human, murine, rat, or of any other origin (including chimeric or humanized antibodies).

(1) Polyclonal antibodies

[0257] Polyclonal antibodies, such as anti-TREM2 polyclonal antibodies, are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (e.g., a purified or recombinant TREM2 protein of the present disclosure) to a protein that is immunogenic in the
species to be immunized, e.g., keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, e.g., maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R¹N=C=NR, where R and R¹ are independently lower alkyl groups. Examples of adjuvants which may be employed include Freund’s complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

[0258] The animals are immunized against the desired antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 µg (for rabbits) or 5 µg (for mice) of the protein or conjugate with 3 volumes of Freund’s complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund’s complete adjuvant by subcutaneous injection at multiple sites. Seven to fourteen days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant-cell culture as protein fusions. Also, aggregating agents such as alum are suitable to enhance the immune response.

(2) Monoclonal antibodies

[0259] Monoclonal antibodies, such as anti-TREM2 monoclonal antibodies, are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translational modifications (e.g., isomerizations, amidations) that may be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies.

[0260] For example, the anti-TREM2 monoclonal antibodies may be made using the hybridoma method first described by Köhler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).
In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization (e.g., a purified or recombinant TREM2 protein of the present disclosure). Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986)).

The immunizing agent will typically include the antigenic protein (e.g., a purified or recombinant TREM2 protein of the present disclosure) or a fusion variant thereof. Generally peripheral blood lymphocytes (“PBLs”) are used if cells of human origin are desired, while spleen or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. Goding, Monoclonal Antibodies: Principles and Practice, Academic Press (1986), pp. 59-103.

Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine or human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which are substances that prevent the growth of HGPRT-deficient-cells.

Preferred immortalized myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors (available from the Salk Institute Cell Distribution Center, San Diego, California USA), as well as SP-2 cells and derivatives thereof (e.g., X63-Ag8-653) (available from the American Type Culture Collection, Manassas, Virginia).
USA). Human myeloma and mouse-human heteromyeloma cell lines have also been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0265] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen (*e.g.*, a TREM2 protein of the present disclosure). Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

[0266] The culture medium in which the hybridoma cells are cultured can be assayed for the presence of monoclonal antibodies directed against the desired antigen (*e.g.*, a TREM2 protein of the present disclosure). Preferably, the binding affinity and specificity of the monoclonal antibody can be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked assay (ELISA). Such techniques and assays are known in the art. For example, binding affinity may be determined by the Scatchard analysis of Munson et al., *Anal. Biochem.*, 107:220 (1980).

[0267] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as tumors in a mammal.

[0268] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, affinity chromatography, and other methods as described above.

[0269] Anti-TREM2 monoclonal antibodies may also be made by recombinant DNA methods, such as those disclosed in U.S. Patent No. 4,816,567, and as described above. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional
procedures (e.g., by using oligonucleotide probes that specifically bind to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host-cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, in order to synthesize monoclonal antibodies in such recombinant host-cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opin. Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Rev.* 130:151-188 (1992).

In certain embodiments, anti-TREM2 antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature*, 348:552-554 (1990). Clackson et al., *Nature*, 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222:581-597 (1991) described the isolation of murine and human antibodies, respectively, from phage libraries. Subsequent publications describe the production of high affinity (nanomolar (“nM”) range) human antibodies by chain shuffling (Marks et al., *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nucl. Acids Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies of desired specificity (e.g., those that bind a TREM2 protein of the present disclosure).

The DNA encoding antibodies or fragments thereof may also be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, et al., *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.
The monoclonal antibodies described herein (e.g., anti-TREM2 antibodies of the present disclosure or fragments thereof) may by monovalent, the preparation of which is well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and a modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues may be substituted with another amino acid residue or are deleted so as to prevent crosslinking. In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

Chimeric or hybrid anti-TREM2 antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptopurine.

(3) **Humanized antibodies**

Anti-TREM2 antibodies of the present disclosure or antibody fragments thereof may further include humanized or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fab, Fab'-SH, Fv, scFv, F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient antibody are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a

Methods for humanizing non-human anti-TREM2 antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanization can be essentially performed following the method of Winter and co-workers, Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-327 (1988); Verhoeyen et al., *Science* 239:1534-1536 (1988), or through substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such “humanized” antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called “best-fit” method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody. Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies. Carter et al., *Proc. Nat’l Acad. Sci. USA* 89:4285 (1992); Presta et al., *J. Immunol.* 151:2623 (1993).
Furthermore, it is important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analyzing the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen or antigens (e.g., TREM2 proteins of the present disclosure), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Various forms of the humanized anti-TREM2 antibody are contemplated. For example, the humanized anti-TREM2 antibody may be an antibody fragment, such as an Fab, which is optionally conjugated with one or more TREM2 ligand, such as HSP60. Alternatively, the humanized anti-TREM2 antibody may be an intact antibody, such as an intact IgG1 antibody.

(4) Human antibodies

Alternatively, human anti-TREM2 antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. The homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Nat'l Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-

**[0280]** Alternatively, phage display technology can be used to produce human anti-TREM2 antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. McCafferty et al., *Nature* 348:552-553 (1990); Hoogenboom and Winter, *J. Mol. Biol.* 227: 381 (1991). According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats, reviewed in, *e.g.*, Johnson, Kevin S. and Chiswell, David J., *Curr. Opin Struct. Biol.* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., *Nature* 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., *J. Mol. Biol.* 222:581-597 (1991), or Griffith et al., EMBO J. 12:725-734 (1993). *See also* U.S. Patent. Nos. 5,565,332 and 5,573,905. Additionally, yeast display technology can be used to produce human anti-TREM2 antibodies and antibody fragments *in vitro* (*e.g.*, WO 2009/036379; WO 2010/105256; WO 2012/009568; US 2009/0181855; US 2010/0056386; and Feldhaus and Siegel (2004) J. Immunological Methods 290:69-80). In other embodiments, ribosome display technology can be used to produce human anti-TREM2 antibodies and antibody fragments *in vitro* (*e.g.*, Roberts and Szostak (1997) Proc Natl Acad Sci 94:12297-12302; Schaffitzel et al. (1999) J. Immunological Methods 231:119-135; Lipovsek and Plückthun (2004) J. Immunological Methods 290:51-67).

**[0281]** The techniques of Cole et al., and Boerner et al., are also available for the preparation of human anti-TREM2 monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer*


[0282] Finally, human anti-TREM2 antibodies may also be generated in vitro by activated B-cells (see U.S. Patent Nos. 5,567,610 and 5,229,275).

(5) Antibody fragments

[0283] In certain embodiments there are advantages to using anti-TREM2 antibody fragments, rather than whole anti-TREM2 antibodies. In some embodiments, smaller fragment sizes allow for rapid clearance and better brain penetration.

[0284] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *J. Biochem. Biophys. Method.* 24:107-117 (1992); and Brennan et al., *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host-cells, for example, using nucleic acids encoding anti-TREM2 antibodies of the present disclosure. Fab, Fv and scFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the straightforward production of large amounts of these fragments. Anti- TREM2 antibody fragments can also be isolated from the antibody phage libraries as discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')2 fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')2 fragments can be isolated directly from recombinant host-cell
culture. Production of Fab and F(ab')₂ antibody fragments with increased *in vivo* half-lives are described in U.S. Patent No. 5,869,046. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Patent No. 5,571,894 and U.S. Patent No. 5,587,458. The anti-TREM2 antibody fragment may also be a “linear antibody,” *e.g.*, as described in U.S. Patent 5,641,870. Such linear antibody fragments may be monospecific or bispecific.

(6) *Bispecific and polyspecific antibodies*

[0285] Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different epitopes, including those on the same or another protein (*e.g.*, one or more TREM2 proteins of the present disclosure). Alternatively, one part of a BsAb can be armed to bind to the target TREM2 antigen, and another can be combined with an arm that binds to a second protein. Such antibodies can be derived from full-length antibodies or antibody fragments (*e.g.*, F(ab')₂ bispecific antibodies).

[0286] Methods for making bispecific antibodies are known in the art. Traditional production of full-length bispecific antibodies is based on the coexpression of two immunoglobulin heavy-chain/light chain pairs, where the two chains have different specificities. Millstein et al., *Nature*, 305:537-539 (1983). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 and in Traunecker et al., *EMBO J.*, 10:3655-3659 (1991).

[0287] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, C₁h₂, and C₁h₃ regions. It is preferred to have the first heavy-chain constant region (C₁h1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if
desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0288] In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only half of the bispecific molecules provides for an easy way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies, see, for example, Suresh et al., Methods in Enzymology 121: 210 (1986); and Garber, Nature Reviews Drug Discovery 13, 799-801 (2014).

[0289] According to another approach described in WO 96/27011 or U.S. Patent No. 5,731,618, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant-cell culture. The preferred interface comprises at least a part of the C{sub H}3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory “cavities” of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0290] Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical
linkage. Brennan et al., *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')2 fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0291] Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.* 175: 217-225 (1992) describes the production of fully humanized bispecific antibody F(ab')2 molecules. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T-cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

[0292] Various techniques for making and isolating bivalent antibody fragments directly from recombinant-cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger et al., *Proc. Nat'l Acad. Sci. USA*, 90: 6444-6448 (1993) has provided an alternative mechanism for making bispecific/bivalent antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific/bivalent antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.*, 152:5368 (1994).
Another method to generate bispecific antibodies is designated controlled Fab-arm exchange (cFAE), which is an easy-to-use method to generate bispecific IgG1 (bsIgG1). The protocol involves the following: (i) separate expression of two parental IgG1s containing single matching point mutations in the CH3 domain; (ii) mixing of parental IgG1s under permissive redox conditions in vitro to enable recombination of half-molecules; (iii) removal of the reductant to allow reoxidation of interchain disulfide bonds; and (iv) analysis of exchange efficiency and final product using chromatography-based or mass spectrometry (MS)-based methods. The protocol generates bsAbs with regular IgG architecture, characteristics and quality attributes both at bench scale (micrograms to milligrams) and at a mini-bioreactor scale (milligrams to grams) that is designed to model large-scale manufacturing (kilograms). Starting from good-quality purified proteins, exchange efficiencies of ≥95% can be obtained within 2-3 days (including quality control). See Labrijn et al., Natur Protocols 9, 2450-2463 (2014); and Garber, Nature Reviews Drug Discovery 13, 799-801 (2014).

Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given molecule (e.g., a TREM2 protein of the present disclosure). In some embodiments a bispecific antibody binds to a first antigen, such as a TREM2 or DAP12 protein of the present disclosure, and a second antigen facilitating transport across the blood-brain barrier. Numerous antigens are known in the art that facilitate transport across the blood-brain barrier (see, e.g., Gabathuler R., Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases, Neurobiol. Dis. 37 (2010) 48-57). Such second antigens include, without limitation, transferrin receptor (TR), insulin receptor (HIR), Insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin receptor, including CRM197 (a non-toxic mutant of diphtheria toxin), llama single domain antibodies such as TMEM 30(A) (Flippase), protein transduction domains such as TAT, Syn-B, or penetratin, polyarginine or generally positively charged peptides, Angiopep peptides such as ANG1005 (see, e.g., Gabathuler, 2010), and other cell surface proteins that are enriched on blood-brain barrier endothelial cells (see, e.g., Daneman et al., PLoS One. 2010 Oct 29;5(10):e13741). In some
embodiments, second antigens for an anti-TREM2 antibody may include, without limitation, a DAP12 antigen of the present disclosure. In other embodiments, second antigens for an anti-DAP12 antibody may include, without limitation, a TREM2 antigen of the present disclosure. In other embodiments, bispecific antibodies that bind to both TREM2 and DAP12 may facilitate and enhance one or more TREM2 activities. In other embodiments, second antigens for an anti-TREM2 antibody may include, without limitation, a beta peptide, antigen or an alpha synuclein protein antigen or, Tau protein antigen or, TDP-43 protein antigen or, prion protein antigen or, huntingtin protein antigen, or RAN, translation products antigen, including the DiPeptide Repeats,(DPRs peptides) composed of glycine-alanine (GA), glycine-proline (GP), glycine-arginine (GR), proline-alanine (PA), or proline-arginine (PR).

(7) Multivalent antibodies

[0296] A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The anti-TREM2 antibodies of the present disclosure or antibody fragments thereof can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g., tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein contains three to about eight, but preferably four, antigen binding sites. The multivalent antibody contains at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain or chains comprise two or more variable domains. For instance, the polypeptide chain or chains may comprise VD1-(X1)n-VD2-(X2)n-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. Similarly, the polypeptide chain or chains may comprise V_H-C_H1-flexible linker-V_H-C_H1-Fc region chain; or V_H-C_H1-V_H-C_H1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent
antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain. The Multivalent antibodies may recognize the Trem2 angigen as well as without limitation additional antigens A beta peptide, antigen or an alpha synuclain protein antigene or, Tau protein antigene or, TDP-43 protein antigene or, prion protein antigene or, huntingtin protein antigene, or RAN, translation Products antigene, including the DiPeptide Repeats,(DPRs peptides) composed of glycine-alanine (GA), glycine-proline (GP), glycine-arginine (GR), proline-alanine (PA), or proline-arginine (PR), Insulin receptor, insulin like growth factor receptor. Transferrin receptor or any other antigen that facilitate antibody transfer across the blood brain barrier.

(8) Effector function engineering

It may also be desirable to modify an anti-TREM2 antibody of the present disclosure to modify effector function and/or to increase serum half-life of the antibody. For example, the Fc receptor binding site on the constant region may be modified or mutated to remove or reduce binding affinity to certain Fc receptors, such as FcγRI, FcγRII, and/or FcγRIII to reduce Antibody-dependent cell-mediated cytotoxicity. In some embodiments, the effector function is impaired by removing N-glycosylation of the Fc region (e.g., in the CH2 domain of IgG) of the antibody. In some embodiments, the effector function is impaired by modifying regions such as 233-236, 297, and/or 327-331 of human IgG as described in PCT WO 99/58572 and Armour et al., Molecular Immunology 40: 585-593 (2003); Reddy et al., J. Immunology 164:1925-1933 (2000). In other embodiments, it may also be desirable to modify an anti-TREM2 antibody of the present disclosure to modify effector function to increase finding selectivity toward the ITIM-containing FcgRIIb (CD32b) to increase clustering of TREM2 antibodies on adjacent cells without activating humoral responses including Antibody-dependent cell-mediated cytotoxicity and antibody-dependent cellular phagocytosis.

[0297] To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term “salvage receptor binding epitope”
refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

(9) *Other amino acid sequence modifications*

[0298] Amino acid sequence modifications of anti-TREM2 antibodies of the present disclosure, or antibody fragments thereof, are also contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibodies or antibody fragments. Amino acid sequence variants of the antibodies or antibody fragments are prepared by introducing appropriate nucleotide changes into the nucleic acid encoding the antibodies or antibody fragments, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics (*i.e.*, the ability to bind or physically interact with a TREM2 protein of the present disclosure). The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

[0299] A useful method for identification of certain residues or regions of the anti-TREM2 antibody that are preferred locations for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells in *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the target antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, alanine scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.
Amino acid sequence insertions include amino- ("N") and/or carboxy- ("C") terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in the Table A below under the heading of “preferred substitutions”. If such substitutions result in a change in biological activity, then more substantial changes, denominated “exemplary substitutions” in Table A, or as further described below in reference to amino acid classes, may be introduced and the products screened.

**TABLE A: Amino Acid Substitutions**

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Exemplary Substitutions</th>
<th>Preferred Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>val; leu; ile</td>
<td>val</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>lys; gln; asn</td>
<td>lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>gln; his; asp; lys; arg</td>
<td>gln</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>glu; asn</td>
<td>glu</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>ser; ala</td>
<td>ser</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>asn; glu</td>
<td>asn</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>asp; gln</td>
<td>asp</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>ala</td>
<td>ala</td>
</tr>
<tr>
<td>His (H)</td>
<td>asn; gln; lys; arg</td>
<td>arg</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>leu; val; met; ala; phe; norleucine</td>
<td>leu</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>norleucine; ile; val; met; ala; phe</td>
<td>ile</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>arg; gln; asn</td>
<td>arg</td>
</tr>
<tr>
<td>Met (M)</td>
<td>leu; phe; ile</td>
<td>leu</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>leu; val; ile; ala; tyr</td>
<td>tyr</td>
</tr>
<tr>
<td>Pro (P)</td>
<td>ala</td>
<td>ala</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>thr</td>
<td>thr</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>Ser</td>
<td>ser</td>
</tr>
<tr>
<td>Original Residue</td>
<td>Exemplary Substitutions</td>
<td>Preferred Substitutions</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>tyr; phe</td>
<td>tyr</td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>trp; phe; thr; ser</td>
<td>phe</td>
</tr>
<tr>
<td>Val (V)</td>
<td>ile; leu; met; phe; ala; norleucine</td>
<td>leu</td>
</tr>
</tbody>
</table>

[0302] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;
(2) neutral hydrophilic: cys, ser, thr;
(3) acidic: asp, glu;
(4) basic: asn, gln, his, lys, arg;
(5) residues that influence chain orientation: gly, pro; and
(6) aromatic: trp, tyr, phe.

[0303] Non-conservative substitutions entail exchanging a member of one of these classes for another class.

[0304] Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment, such as an Fv fragment).

[0305] A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human anti-TREM2 antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g., 6-7 sites) are mutated to generate
all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding.

Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen (e.g., a TREM2 protein of the present disclosure). Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0306] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

[0307] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0308] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the
addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0309] Nucleic acid molecules encoding amino acid sequence variants of the anti-IgE antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibodies (e.g., anti-TREM2 antibodies of the present disclosure) or antibody fragments.

(10) Other antibody modifications

[0310] Anti-TREM2 antibodies of the present disclosure, or antibody fragments thereof, can be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available, or to contain different types of drug conjugates that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc. Such techniques and other suitable

[0311] Drug conjugation involves coupling of a biological active cytotoxic (anticancer) payload or drug to an antibody that specifically targets a certain tumor marker (e.g., a protein that, ideally, is only to be found in or on tumor cells). Antibodies track these proteins down in the body and attach themselves to the surface of cancer cells. The biochemical reaction between the antibody and the target protein (antigen) triggers a signal in the tumor cell, which then absorbs or internalizes the antibody together with the cytotoxin. After the ADC is internalized, the cytotoxic drug is released and kills the cancer. Due to this targeting, ideally the drug has lower side effects and gives a wider therapeutic window than other chemotherapeutic agents. Technics to conjugate antibodies are disclosed are known in the art (see, e.g., Jane de Lartigue, OncLive July 5, 2012; ADC Review on antibody-drug conjugates; and Ducry et al., (2010). *Bioconjugate Chemistry* 21 (1): 5–13).

*Binding assays and other assays*

[0312] Anti-TREM2 antibodies of the present disclosure may be tested for antigen binding activity, *e.g.*, by known methods such as ELISA, Western blot, *etc.*

[0313] In some embodiments, competition assays may be used to identify an antibody that competes with any of the antibodies listed in Table 1, selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87, and/or human and/or humanized MAB17291 for binding to TREM2. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by any of the antibodies listed in Table 1, selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23,

[0314] In an exemplary competition assay, immobilized TREM2 or cells expressing TREM2 on cell surface are incubated in a solution comprising a first labeled antibody that binds to TREM2 (e.g., human or non-human primate) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to TREM2. The second antibody may be present in a hybridoma supernatant. As a control, immobilized TREM2 or cells expressing TREM2 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to TREM2, excess unbound antibody is removed, and the amount of label associated with immobilized TREM2 or cells expressing TREM2 is measured. If the amount of label associated with immobilized TREM2 or cells expressing TREM2 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to TREM2. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Nucleic acids, vectors, and host cells

[0315] Anti-TREM2 antibodies of the present disclosure may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In some embodiments, isolated nucleic acids having a nucleotide sequence encoding any of the anti-TREM2 antibodies of the present disclosure are provided. Such nucleic acids may encode an amino acid sequence containing the VL and/or an amino acid sequence containing the VH of the
anti-TREM2 antibody (e.g., the light and/or heavy chains of the antibody). In some embodiments, one or more vectors (e.g., expression vectors) containing such nucleic acids are provided. In some embodiments, a host cell containing such nucleic acid is also provided. In some embodiments, the host cell contains (e.g., has been transduced with): (1) a vector containing a nucleic acid that encodes an amino acid sequence containing the VL of the antibody and an amino acid sequence containing the VH of the antibody, or (2) a first vector containing a nucleic acid that encodes an amino acid sequence containing the VL of the antibody and a second vector containing a nucleic acid that encodes an amino acid sequence containing the VH of the antibody. In some embodiments, the host cell is eukaryotic, e.g., a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). Host cells of the present disclosure also include, without limitation, isolated cells, in vitro cultured cells, and ex vivo cultured cells.

[0316] Methods of making an anti-TREM2 antibody of the present disclosure are provided. In some embodiments, the method includes culturing a host cell of the present disclosure containing a nucleic acid encoding the anti-TREM2 antibody, under conditions suitable for expression of the antibody. In some embodiments, the antibody is subsequently recovered from the host cell (or host cell culture medium).

[0317] For recombinant production of an anti-TREM2 antibody of the present disclosure, a nucleic acid encoding the anti-TREM2 antibody is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0318] Suitable vectors containing a nucleic acid sequence encoding any of the anti-TREM2 antibodies of the present disclosure, or fragments thereof polypeptides (including antibodies) described herein include, without limitation, cloning vectors and expression vectors. Suitable cloning vectors can be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may
carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, *e.g.*, pUC18, pUC19, Bluescript (*e.g.*, pBS SK+) and its derivatives, mpl8, mpl9, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Stratagene, and Invitrogen.

Expression vectors generally are replicable polynucleotide constructs that contain a nucleic acid of the present disclosure. The expression vector may replicate in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (*i.e.*, translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

The vectors containing the nucleic acids of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell. In some embodiments, the vector contains a nucleic acid containing one or more amino acid sequences encoding an anti-TREM2 antibody of the present disclosure.

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells. For example, anti-TREM2 antibodies of the present disclosure may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria (*e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523; and Charlton, *Methods in Molecular Biology, Vol.*
248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*). After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0322] In addition to prokaryotes, eukaryotic microorganisms, such as filamentous fungi or yeast, are also suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized,” resulting in the production of an antibody with a partially or fully human glycosylation pattern (e.g., Gemgros, *Nat. Biotech.* 22:1409-1414 (2004); and Li et al., *Nat. Biotech.* 24:210-215 (2006)).

[0323] Suitable host cells for the expression of glycosylated antibody can also be derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts (e.g., U.S. Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429, describing PLANTIBODIES™ technology for producing antibodies in transgenic plants.).

[0324] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu,

**Pharmaceutical compositions**

[0325] Anti-TREM2 antibodies of the present disclosure can be incorporated into a variety of formulations for therapeutic administration by combining the antibodies with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms. Examples of such formulations include, without limitation, tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Pharmaceutical compositions can include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers of diluents, which are vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents include, without limitation, distilled water, buffered water, physiological saline, PBS, Ringer’s solution, dextrose solution, and Hank’s solution. A pharmaceutical composition or formulation of the present disclosure can further include other carriers, adjuvants, or non-toxic, nontherapeutic, nonimmunogenic stabilizers, excipients and the like. The compositions can also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents and detergents.

[0326] A pharmaceutical composition of the present disclosure can also include any of a variety of stabilizing agents, such as an antioxidant for example. When the pharmaceutical composition includes a polypeptide, the polypeptide can be complexed with various well-known compounds that enhance the *in vivo* stability of the polypeptide, or otherwise enhance its pharmacological properties (*e.g.*, increase the half-life of the polypeptide, reduce its toxicity, and enhance solubility or uptake). Examples of such modifications or complexing agents include, without limitation, sulfate, gluconate, citrate and phosphate. The polypeptides of a composition can also be complexed with molecules that enhance their *in vivo* attributes. Such molecules
include, without limitation, carbohydrates, polyamines, amino acids, other peptides, ions (e.g., sodium, potassium, calcium, magnesium, manganese), and lipids.


[0328] For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, and edible white ink. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

[0329] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0330] The components used to formulate the pharmaceutical compositions are preferably of high purity and are substantially free of potentially harmful contaminants (e.g., at least National Food (NF) grade, generally at least analytical grade, and more typically at least pharmaceutical
grade). Moreover, compositions intended for *in vivo* use are usually sterile. To the extent that a given compound must be synthesized prior to use, the resulting product is typically substantially free of any potentially toxic agents, particularly any endotoxins, which may be present during the synthesis or purification process. Compositions for parental administration are also sterile, substantially isotonic and made under GMP conditions.

[0331] Formulations may be optimized for retention and stabilization in the brain or central nervous system. When the agent is administered into the cranial compartment, it is desirable for the agent to be retained in the compartment, and not to diffuse or otherwise cross the blood brain barrier. Stabilization techniques include cross-linking, multimerizing, or linking to groups such as polyethylene glycol, polyacrylamide, neutral protein carriers, *etc.* in order to achieve an increase in molecular weight.

[0332] Other strategies for increasing retention include the entrapment of the antibody, such as an anti-TREM2 antibody of the present disclosure, in a biodegradable or bioerodible implant. The rate of release of the therapeutically active agent is controlled by the rate of transport through the polymeric matrix, and the biodegradation of the implant. The transport of drug through the polymer barrier will also be affected by compound solubility, polymer hydrophilicity, extent of polymer cross-linking, expansion of the polymer upon water absorption so as to make the polymer barrier more permeable to the drug, geometry of the implant, and the like. The implants are of dimensions commensurate with the size and shape of the region selected as the site of implantation. Implants may be particles, sheets, patches, plaques, fibers, microcapsules and the like and may be of any size or shape compatible with the selected site of insertion.

[0333] The implants may be monolithic, *i.e.* having the active agent homogenously distributed through the polymeric matrix, or encapsulated, where a reservoir of active agent is encapsulated by the polymeric matrix. The selection of the polymeric composition to be employed will vary with the site of administration, the desired period of treatment, patient tolerance, the nature of the disease to be treated and the like. Characteristics of the polymers will include biodegradability at the site of implantation, compatibility with the agent of interest, ease of encapsulation, a half-life in the physiological environment.
Biodegradable polymeric compositions which may be employed may be organic esters or ethers, which when degraded result in physiologically acceptable degradation products, including the monomers. Anhydrides, amides, orthoesters or the like, by themselves or in combination with other monomers, may find use. The polymers will be condensation polymers. The polymers may be cross-linked or non-cross-linked. Of particular interest are polymers of hydroxylaliphatic carboxylic acids, either homo- or copolymers, and polysaccharides. Included among the polyesters of interest are polymers of D-lactic acid, L-lactic acid, racemic lactic acid, glycolic acid, polycaprolactone, and combinations thereof. By employing the L-lactate or D-lactate, a slowly biodegrading polymer is achieved, while degradation is substantially enhanced with the racemate. Copolymers of glycolic and lactic acid are of particular interest, where the rate of biodegradation is controlled by the ratio of glycolic to lactic acid. The most rapidly degraded copolymer has roughly equal amounts of glycolic and lactic acid, where either homopolymer is more resistant to degradation. The ratio of glycolic acid to lactic acid will also affect the brittleness of in the implant, where a more flexible implant is desirable for larger geometries. Among the polysaccharides of interest are calcium alginate, and functionalized celluloses, particularly carboxymethylcellulose esters characterized by being water insoluble, a molecular weight of about 5 kD to 500 kD, etc. Biodegradable hydrogels may also be employed in the implants of the subject invention. Hydrogels are typically a copolymer material, characterized by the ability to imbibe a liquid. Exemplary biodegradable hydrogels which may be employed are described in Heller in: Hydrogels in Medicine and Pharmacy, N. A. Peppes ed., Vol. III, CRC Press, Boca Raton, Fla., 1987, pp 137-149.

Pharmaceutical dosages

Pharmaceutical compositions of the present disclosure containing an anti-TREM2 antibody of the present disclosure may be administered to an individual in need of treatment with the anti-TREM2 antibody, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, intracranial, intraspinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.
Dosages and desired drug concentration of pharmaceutical compositions of the present disclosure may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles described in Mordenti, J. and Chappell, W. “The Use of Interspecies Scaling in Toxicokinetics,” In Toxicokinetics and New Drug Development, Yacobi et al., Eds, Pergamon Press, New York 1989, pp.42-46.

For in vivo administration of any of the anti-TREM2 antibodies of the present disclosure, normal dosage amounts may vary from about 10 ng/kg up to about 100 mg/kg of an individual’s body weight or more per day, preferably about 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration. For repeated administrations over several days or longer, depending on the severity of the disease, disorder, or condition to be treated, the treatment is sustained until a desired suppression of symptoms is achieved.

An exemplary dosing regimen may include administering an initial dose of an anti-TREM2 antibody, of about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg every other week. Other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the physician wishes to achieve. For example, dosing an individual from one to twenty-one times a week is contemplated herein. In certain embodiments, dosing ranging from about 3 µg/kg to about 2 mg/kg (such as about 3 µg/kg, about 10 µg/kg, about 30 µg/kg, about 100 µg/kg, about 300 µg/kg, about 1 mg/kg, and about 2 mg/kg) may be used. In certain embodiments, dosing frequency is three times per day, twice per day, once per day, once every other day, once weekly, once every two weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, or once monthly, once every two months, once every three months, or longer. Progress of the therapy is easily monitored by conventional techniques and assays. The dosing regimen, including the anti-TREM2 antibody administered, can vary over time independently of the dose used.
Dosages for a particular anti-TREM2 antibody may be determined empirically in individuals who have been given one or more administrations of the anti-TREM2 antibody. Individuals are given incremental doses of an anti-TREM2 antibody. To assess efficacy of an anti-TREM2 antibody, a clinical symptom of any of the diseases, disorders, or conditions of the present disclosure (e.g., dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, and multiple sclerosis) can be monitored.

Administration of an anti-TREM2 antibody of the present disclosure can be continuous or intermittent, depending, for example, on the recipient’s physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an anti-TREM2 antibody may be essentially continuous over a preselected period of time or may be in a series of spaced doses.

Guidance regarding particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Patent Nos. 4,657,760; 5,206,344; or 5,225,212. It is within the scope of the invention that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Therapeutic uses

Further aspects of the present disclosure provide methods for modulating (e.g., activating or inhibiting) TREM2, modulating (e.g., activating or inhibiting) DAP12, modulating (e.g., activating or inhibiting) PI3K, modulating (e.g., increasing or reducing) expression of one or more cytokines (e.g., IL-12p70, IL-6, and IL-10), or modulating (e.g., increasing or reducing) expression of one or more pro-inflammatory mediators (e.g., L-1β and TNF) in an individual in need thereof, by administering to the individual a therapeutically effective amount of an anti-
TREM2 antibody of the present disclosure to modulate (e.g., induce or inhibit) one or more TREM2 activities in the individual.

[0343] As disclosed herein, anti-TREM2 antibodies of the present disclosure may be used for preventing, reducing risk, or treating dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, amyotrophic lateral sclerosis, Huntington’s disease, Taapathy disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma, lupus, acute and chronic colitis, wound healing, Crohn’s disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet's disease, Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear palsy, cortical basal ganglionic degeneration, acute disseminated encephalomyelitis, granulomatous disorders, Sarcoidosis, diseases of aging, seizures, spinal cord injury, traumatic brain injury, age related macular degeneration, glaucoma, retinitis pigmentosa, retinal degeneration, respiratory tract infection, sepsis, eye infection, systemic infection, lupus, arthritis, multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrotic disease, Paget’s disease of bone, and/or, cancer (e.g., bladder cancer breast cancer, colon and rectal cancer, endometrial cancer, kidney cancer, renal cell cancer, renal pelvis cancer, leukemia, lung cancer, melanoma, non-Hodgkin’s lymphoma, pancreatic cancer, prostate cancer, ovarian cancer, fibrosarcoma, and thyroid cancer). In some embodiments, the anti-TREM2 antibodies are agonist antibodies. In some embodiments, the anti-TREM2 antibodies are inert antibodies. In some embodiments, the anti-TREM2 antibodies are antagonist antibodies.

[0344] In some embodiments, the present disclosure provides methods of preventing, reducing risk, or treating an individual having dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, amyotrophic lateral sclerosis, Huntington’s disease, Taapathy disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma, lupus, acute and chronic colitis, wound healing, Crohn's disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet's disease, Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear
palsy, cortical basal ganglionic degeneration, acute disseminated encephalomyelitis, granulomatous disorders, Sarcoidosis, diseases of aging, seizures, spinal cord injury, traumatic brain injury, age related macular degeneration, glaucoma, retinitis pigmentosa, retinal degeneration, respiratory tract infection, sepsis, eye infection, systemic infection, lupus, arthritis, multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrosic disease, Paget's disease of bone, and cancer, by administering to the individual a therapeutically effective amount of an anti-TREM2 antibody of the present disclosure. In some embodiments, the anti-TREM2 antibody is an agonist antibody. In some embodiments, the anti-TREM2 antibody is an inert antibody. In some embodiments, the anti-TREM2 antibody is an antagonist antibody. In certain embodiments, the individual has a heterozygous TREM2 variant allele having an glutamic acid to stop codon substitution in the nucleic acid sequence encoding amino acid residue 14 of the human TREM2 protein (SEQ ID NO: 1). In certain embodiments, the individual has a heterozygous TREM2 variant allele having a glutamine to stop codon substitution in the nucleic acid sequence encoding amino acid residue 33 of the human TREM2 protein (SEQ ID NO: 1). In certain embodiments, the individual has a heterozygous TREM2 variant allele having a tryptophan to stop codon substitution in the nucleic acid sequence encoding amino acid residue 44 of the human TREM2 protein (SEQ ID NO: 1). In certain embodiments, the individual has a heterozygous TREM2 variant allele having an arginine to histidine amino acid substitution at amino acid residue 47 of the human TREM2 protein (SEQ ID NO: 1). In certain embodiments, the individual has a heterozygous TREM2 variant allele having a tryptophan to stop codon substitution in the nucleic acid sequence encoding amino acid residue 78 of the human TREM2 protein (SEQ ID NO: 1). In certain embodiments, the individual has a heterozygous TREM2 variant allele having a valine to glycine amino acid substitution at an amino acid corresponding to amino acid residue 126 of the human TREM2 protein (SEQ ID NO: 1). In certain embodiments, the individual has a heterozygous TREM2 variant allele having an aspartic acid to glycine amino acid substitution at an amino acid corresponding to amino acid residue 134 of the human TREM2 protein (SEQ ID NO: 1). In certain embodiments, the individual has a heterozygous TREM2 variant allele having a lysine to asparagine amino acid substitution at an
amino acid corresponding to amino acid residue 186 of the human TREM2 protein (SEQ ID NO: 1).

[0345] In some embodiments, the individual has a heterozygous TREM2 variant allele having a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G313 of the nucleic acid sequence encoding SEQ ID NO: 1; a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G267 of the nucleic acid sequence encoding SEQ ID NO: 1; a threonine to methionine amino acid substitution at an amino acid corresponding to amino acid residue Thr66 of SEQ ID NO: 1; and/or a serine to cysteine amino acid substitution at an amino acid corresponding to amino acid residue Ser116 of SEQ ID NO: 1.

[0346] In some embodiments, the individual has a heterozygous DAP12 variant allele having a methionine to threonine substitution at an amino acid corresponding to amino acid residue Met1 of SEQ ID NO: 2, a glycine to arginine amino acid substitution at an amino acid corresponding to amino acid residue Gly49 of SEQ ID NO: 2, a deletion within exons 1-4 of the nucleic acid sequence encoding SEQ ID NO: 2, an insertion of 14 amino acid residues at exon 3 of the nucleic acid sequence encoding SEQ ID NO: 2, and/or a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G141 of the nucleic acid sequence encoding SEQ ID NO: 2.

[0347] As disclosed herein, anti-TREM2 antibodies of the present disclosure may also be used for inducing and/or promoting innate immune cell survival. In some embodiments, the present disclosure provides methods of inducing or promoting innate immune cell survival an individual in need thereof, by administering to the individual a therapeutically effective amount of an agonist anti-TREM2 antibody of the present disclosure.

[0348] As disclosed herein, anti-TREM2 antibodies of the present disclosure may also be used for inducing and/or promoting wound healing, such as after injury. In some embodiments, the wound healing may be colonic wound repair following injury. In some embodiments, the present disclosure provides methods of inducing or promoting wound healing an individual in need thereof, by administering to the individual a therapeutically effective amount of an agonist anti-TREM2 antibody of the present disclosure.
[0349] In some embodiments, the methods of the present disclosure may involve the coadministration of anti-TREM2 antibodies, or bispecific antibodies with TLR antagonists or with agents neutralizing TLR agonist (e.g., neutralizing cytokine or interleukin antibodies).

[0350] In some embodiments, the methods of the present disclosure may involve the administration of chimeric constructs, including an anti-TREM2 antibody of the present disclosure in conjunction with a TREM2 ligand, such as HSP60.

**Dementia**

[0351] Dementia is a non-specific syndrome (i.e., a set of signs and symptoms) that presents as a serious loss of global cognitive ability in a previously unimpaired person, beyond what might be expected from normal ageing. Dementia may be static as the result of a unique global brain injury. Alternatively, dementia may be progressive, resulting in long-term decline due to damage or disease in the body. While dementia is much more common in the geriatric population, it can also occur before the age of 65. Cognitive areas affected by dementia include, without limitation, memory, attention span, language, and problem solving. Generally, symptoms must be present for at least six months to before an individual is diagnosed with dementia.

[0352] Exemplary forms of dementia include, without limitation, frontotemporal dementia, Alzheimer's disease, vascular dementia, semantic dementia, and dementia with Lewy bodies.

[0353] Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat dementia. In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having dementia (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

**Frontotemporal dementia**

[0354] Frontotemporal dementia (FTD) is a condition resulting from the progressive deterioration of the frontal lobe of the brain. Over time, the degeneration may advance to the

A substantial portion of FTD cases are inherited in an autosomal dominant fashion, but even in one family, symptoms can span a spectrum from FTD with behavioral disturbances, to Primary Progressive Aphasia, to Cortico-Basal Ganglionic Degeneration. FTD, like most neurodegenerative diseases, can be characterized by the pathological presence of specific protein aggregates in the diseased brain. Historically, the first descriptions of FTD recognized the presence of intraneuronal accumulations of hyperphosphorylated Tau protein in neurofibrillary tangles or Pick bodies. A causal role for the microtubule associated protein Tau was supported by the identification of mutations in the gene encoding the Tau protein in several families (Hutton, M., et al., Nature 393:702-705 (1998). However, the majority of FTD brains show no accumulation of hyperphosphorylated Tau but do exhibit immunoreactivity to ubiquitin (Ub) and TAR DNA binding protein (TDP43) (Neumann, M., et al., Arch. Neurol. 64:1388-1394 (2007)). A majority of those FTD cases with Ub inclusions (FTD-U) were shown to carry mutations in the progranulin gene.

Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat FTD. In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having FTD (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

Alzheimer's disease

Alzheimer’s disease (AD) is the most common form of dementia. There is no cure for the disease, which worsens as it progresses, and eventually leads to death. Most often, AD is
diagnosed in people over 65 years of age. However, the less-prevalent early-onset Alzheimer's can occur much earlier.

[0358] Common symptoms of Alzheimer’s disease include, behavioral symptoms, such as difficulty in remembering recent events; cognitive symptoms, confusion, irritability and aggression, mood swings, trouble with language, and long-term memory loss. As the disease progresses bodily functions are lost, ultimately leading to death. Alzheimer’s disease develops for an unknown and variable amount of time before becoming fully apparent, and it can progress undiagnosed for years.

[0359] Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat Alzheimer’s disease. In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having Alzheimer’s disease (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

Nasu-Hakola disease

[0360] Nasu-Hakola disease (NHD), which may alternatively be referred to as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOS), is a rare inherited leukodystrophy characterized by progressive presenile dementia associated with recurrent bone fractures due to polycystic osseous lesions of the lower and upper extremities. NHD disease course is generally divided into four stages: latent, osseous, early neurologic, and late neurologic. After a normal development during childhood (latent stage), NHD starts manifesting during adolescence or young adulthood (typical age of onset 20-30 years) with pain in the hands, wrists, ankles, and feet. Patients then start suffering from recurrent bone fractures due to polycystic osseous and osteoporotic lesions in the limb bones (osseous stage). During the third or fourth decade of life (early neurologic stage), patients present with pronounced personality changes (e.g., euphoria, lack of concentration, loss of judgment, and social inhibitions) characteristic of a frontal lobe syndrome. Patients also typically suffer from progressive memory disturbances. Epileptic seizures are also frequently observed. Finally (late
neurologic stage), patients progress to a profound dementia, are unable to speak and move, and usually die by the age of 50.

Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat Nasu-Hakola disease (NHD). In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having NHD (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

*Parkinson's disease*

Parkinson’s disease, which may be referred to as idiopathic or primary parkinsonism, hypokinetic rigid syndrome (HRS), or paralysis agitans, is a neurodegenerative brain disorder that affects motor system control. The progressive death of dopamine-producing cells in the brain leads to the major symptoms of Parkinson’s. Most often, Parkinson’s disease is diagnosed in people over 50 years of age. Parkinson’s disease is idiopathic (having no known cause) in most people. However, genetic factors also play a role in the disease.

Symptoms of Parkinson’s disease include, without limitation, tremors of the hands, arms, legs, jaw, and face, muscle rigidity in the limbs and trunk, slowness of movement (bradykinesia), postural instability, difficulty walking, neuropsychiatric problems, changes in speech or behavior, depression, anxiety, pain, psychosis, dementia, hallucinations, and sleep problems.

Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat Parkinson’s disease. In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having Parkinson’s disease (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).
Amyotrophic lateral sclerosis

[0365] As used herein, amyotrophic lateral sclerosis (ALS) or, motor neuron disease or, Lou Gehrig’s disease are used interchangeably and refer to a debilitating disease with varied etiology characterized by rapidly progressive weakness, muscle atrophy and fasciculations, muscle spasticity, difficulty speaking (dysarthria), difficulty swallowing (dysphagia), and difficulty breathing (dyspnea).

[0366] It has been shown that progranulin play a role in ALS (Schymick, JC et al., (2007) J Neurol Neurosurg Psychiatry;78:754-6) and protects against the damage caused by ALS causing proteins such as TDP-43 (Laird, AS et al., (2010). PLoS ONE 5: e13368). It was also demonstrated that pro-NGF induces p75 mediated death of oligodendrocytes and corticospinal neurons following spinal cord injury (Beatty et al., Neuron (2002),36, pp. 375-386; Giehl et al, Proc. Natl. Acad. Sci USA (2004), 101, pp 6226-30).

[0367] Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat ALS. In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having ALS (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

Huntington’s disease

[0368] Huntington’s disease (HD) is an inherited neurodegenerative disease caused by an autosomal dominant mutation in the Huntingtin gene (HTT). Expansion of a cytokine-adenine-guanine (CAG) triplet repeat within the Huntingtin gene results in production of a mutant form of the Huntingtin protein (Htt) encoded by the gene. This mutant Huntingtin protein (mHtt) is toxic and contributes to neuronal death. Symptoms of Huntington’s disease most commonly appear between the ages of 35 and 44, although they can appear at any age.

[0369] Symptoms of Huntington’s disease, include, without limitation, motor control problems, jerky, random movements (chorea), abnormal eye movements, impaired balance, seizures, difficulty chewing, difficulty swallowing, cognitive problems, altered speech, memory
deficits, thinking difficulties, insomnia, fatigue, dementia, changes in personality, depression, anxiety, and compulsive behavior.

[0370] Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat Huntington’s disease (HD). In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having HD (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).

*Taupathy disease*

[0371] Taupathy diseases, or Tauopathies, are a class of neurodegenerative disease caused by aggregation of the microtubule-associated protein tau within the brain. Alzheimer’s disease (AD) is the most well-known Taupathy disease, and involves an accumulation of tau protein within neurons in the form of insoluble neurofibrillary tangles (NFTs). Other Taupathy diseases and disorders include progressive supranuclear palsy, dementia pugilistica (chronic traumatic encephalopathy), Frontotemporal dementia and parkinsonism linked to chromosome 17, Lytico-Bodig disease (Parkinson-dementia complex of Guam), Tangle-predominant dementia, Ganglioglioma and gangliocytoma, Meningioangiomatosis, Subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis, Pick’s disease, corticobasal degeneration, Argyrophilic grain disease (AGD), Huntington’s disease, frontotemporal dementia, and frontotemporal lobar degeneration.

[0372] Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat Taupathy disease. In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having Taupathy disease (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, or reduced expression of one or more pro-inflammatory mediators).
Multiple sclerosis

Multiple sclerosis (MS) can also be referred to as disseminated sclerosis or encephalomyelitis disseminata. MS is an inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged, leading to demyelination and scarring as well as a broad spectrum of signs and symptoms. MS affects the ability of nerve cells in the brain and spinal cord to communicate with each other effectively. Nerve cells communicate by sending electrical signals called action potentials down long fibers called axons, which are contained within an insulating substance called myelin. In MS, the body’s own immune system attacks and damages the myelin. When myelin is lost, the axons can no longer effectively conduct signals. MS onset usually occurs in young adults, and is more common in women.

Symptoms of MS include, without limitation, changes in sensation, such as loss of sensitivity or tingling; pricking or numbness, such as hypoesthesia and paresthesia; muscle weakness; clonus; muscle spasms; difficulty in moving; difficulties with coordination and balance, such as ataxia; problems in speech, such as dysarthria, or in swallowing, such as dysphagia; visual problems, such as nystagmus, optic neuritis including phosphenes, and diplopia; fatigue; acute or chronic pain; and bladder and bowel difficulties; cognitive impairment of varying degrees; emotional symptoms of depression or unstable mood; Uhthoff’s phenomenon, which is an exacerbation of extant symptoms due to an exposure to higher than usual ambient temperatures; and Lhermitte’s sign, which is an electrical sensation that runs down the back when bending the neck.

Without wishing to be bound by theory, it is believed that administering an anti-TREM2 antibody of the present disclosure can prevent, reduce the risk, and/or treat multiple sclerosis. In some embodiments, administering an anti-TREM2 antibody may induce one or more TREM2 activities in an individual having multiple sclerosis (e.g., DAP12 phosphorylation, PI3K activation, increased expression of one or more anti-inflammatory mediators, and reduced expression of one or more pro-inflammatory mediators).
Cancer

Yet further aspects of the present disclosure provide methods for preventing, reducing risk, or treating an individual having cancer, comprising administering to the individual a therapeutically effective amount of an isolated anti-TREM2 antibody of the present disclosure. Any of the isolated antibodies of the present disclosure may be used in these methods. In some embodiments, the isolated antibody is an agonist antibody of the present disclosure. In other embodiments, the isolated antibody is an antagonist antibody of the present disclosure.

As described above, the tumor microenvironment is known to contain a heterogeneous immune infiltrate, which includes T lymphocytes, macrophages and cells of myeloid/granulocytic lineage. In particular, the presence of M2-macrophages in tumors is associated with poor prognosis. Therapies that reduce the number of these cells in the tumor, such as CSF1R blocking agents, are showing beneficial effects in preclinical models and early stage clinical studies. It has been shown that TREM2 synergizes with CSF1 to promote survival of macrophages in vitro, and that this effect is particularly prominent in M2-type macrophages, compared to other types of phagocytic cells. A seminal preclinical study has also shown synergies between drugs that target tumor-associated macrophages (e.g., CSF1/CSF1R blocking antibodies) and checkpoint blocking antibodies that target T cells, indicating that manipulating both cell types shows efficacy in tumor models where individual therapies are poorly effective (Zhu Y; Cancer Res. 2014 Sep 15; 74(18):5057-69). Therefore, without wishing to be bound by theory, it is thought that blocking TREM2 signaling in tumor associated macrophages may inhibit suppression of the immune response in the tumor microenvironment, resulting in a therapeutic anti-tumor immune response.

Due to the synergies between TREM2 and CSF1, and between targeting tumor-associated macrophages and targeting T cells, in some embodiments, the methods for preventing, reducing risk, or treating an individual having cancer further include administering to the individual at least one antibody that specifically binds to an inhibitory checkpoint molecule. Examples of antibodies that specifically bind to an inhibitory checkpoint molecule include, without limitation, an anti-PD-L1 antibody, an anti-CTLA4 antibody, an anti-PD-L2 antibody, an
anti-PD-1 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, and anti-HVEM antibody, an anti-BTLA antibody, an anti-GAL9 antibody, an anti-TIM3 antibody, an anti-A2AR antibody, an anti-LAG-3 antibody, an anti-phosphatidylserine antibody, and any combination thereof. In some embodiments, the at least one antibody that specifically binds to an inhibitory checkpoint molecule is administered in combination with an antagonist anti-TREM2 antibody of the present disclosure.

[0379] In some embodiments, a cancer to be prevented or treated by the methods of the present disclosure includes, but is not limited to, squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In some embodiments, the cancer is colorectal cancer. In some embodiments, the cancer is selected from non-small cell lung cancer, glioblastoma, neuroblastoma, renal cell carcinoma, bladder cancer, ovarian cancer, melanoma, breast carcinoma, gastric cancer, and hepatocellular carcinoma. In some embodiments, the cancer is triple-negative breast carcinoma. In some embodiments, the cancer may be an early stage cancer or a late stage cancer. In some embodiments, the cancer may be a primary tumor. In some embodiments, the cancer may be a metastatic tumor at a second site derived from any of the above types of cancer.
In some embodiments, anti-TREM2 antibodies of the present disclosure may be used for preventing, reducing risk, or treating cancer, including, without limitation, bladder cancer, breast cancer, colon and rectal cancer, endometrial cancer, kidney cancer, renal cell cancer, renal pelvis cancer, leukemia, lung cancer, melanoma, non-Hodgkin’s lymphoma, pancreatic cancer, prostate cancer, ovarian cancer, fibrosarcoma, and thyroid cancer.

In some embodiments, the present disclosure provides methods of preventing, reducing risk, or treating an individual having cancer, by administering to the individual a therapeutically effective amount of an anti-TREM2 antibody of the present disclosure.

In some embodiments, the method further includes administering to the individual at least one antibody that specifically binds to an inhibitory checkpoint molecule, and/or another standard or investigational anti-cancer therapy. In some embodiments, the at least one antibody that specifically binds to an inhibitory checkpoint molecule is administered in combination with the isolated antibody. In some embodiments, the at least one antibody that specifically binds to an inhibitory checkpoint molecule is selected from an anti-PD-L1 antibody, an anti-CTLA4 antibody, an anti-PD-L2 antibody, an anti-PD-1 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, and anti-HVEM antibody, an anti- B- and T-lymphocyte attenuator (BTLA) antibody, an anti- Killer inhibitory receptor (KIR) antibody, an anti-GAL9 antibody, an anti-TIM3 antibody, an anti-A2AR antibody, an anti-LAG-3 antibody, an anti-phosphatidylerserine antibody, an anti-CD27 antibody, and any combination thereof. In some embodiments, the standard or investigational anti-cancer therapy is one or more therapies selected from radiotherapy, cytotoxic chemotherapy, targeted therapy, imatinib (Gleevec®), trastuzumab (Herceptin®), adoptive cell transfer (ACT), chimeric antigen receptor T cell transfer (CAR-T), vaccine therapy, and cytokine therapy.

In some embodiments, the method further includes administering to the individual at least one antibody that specifically binds to an inhibitory cytokine. In some embodiments, the at least one antibody that specifically binds to an inhibitory cytokine is administered in combination with the isolated antibody. In some embodiments, the at least one antibody that specifically
binds to an inhibitory cytokine is selected from an anti-CCL2 antibody, an anti-CSF-1 antibody, an anti-IL-2 antibody, and any combination thereof.

[0384] In some embodiments, the method further includes administering to the individual at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein. In some embodiments, the at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein is administered in combination with the isolated antibody. In some embodiments, the at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein is selected from an agonist anti-CD40 antibody, an agonist anti-OX40 antibody, an agonist anti-ICOS antibody, an agonist anti-CD28 antibody, an agonist anti-CD137/4-1BB antibody, an agonist anti-CD27 antibody, an agonist anti-glucocorticoid-induced TNFR-related protein GITR antibody, and any combination thereof.

[0385] In some embodiments, the method further includes administering to the individual at least one stimulatory cytokine. In some embodiments, the at least one stimulatory cytokine is administered in combination with the isolated antibody. In some embodiments, the at least one stimulatory cytokine is selected from TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL20 family member, IL-33, LIF, OSM, CNTF, TGF-beta, IL-11, IL-12, IL-17, IL-8, CRP, IFN-α, IFN-β, IL-2, IL-18, GM-CSF, G-CSF, and any combination thereof.

Kits/Articles of Manufacture

[0386] The present disclosure also provides kits containing an isolated antibody of the present disclosure (e.g., an anti-TREM2 or anti-DAP12 antibody described herein), or a functional fragment thereof. Kits of the present disclosure may include one or more containers comprising a purified antibody of the present disclosure. In some embodiments, the kits further include instructions for use in accordance with the methods of this disclosure. In some embodiments, these instructions comprise a description of administration of the isolated antibody of the present disclosure (e.g., an anti-TREM2 or anti-DAP12 antibody described herein) to prevent, reduce risk, or treat an individual having a disease, disorder, or injury selected from dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, multiple sclerosis, and cancer, according to any methods of this disclosure.
[0387] In some embodiments, the instructions comprise a description of how to detect TREM2 and/or DAP12, for example in an individual, in a tissue sample, or in a cell. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the disease and the stage of the disease.

[0388] In some embodiments, the kits may further include another antibody of the present disclosure (e.g., at least one antibody that specifically binds to an inhibitory checkpoint molecule, at least one antibody that specifically binds to an inhibitory cytokine, and/or at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein) and/or at least one stimulatory cytokine. In some embodiments, the kits may further include instructions for using the antibody and/or stimulatory cytokine in combination with an isolated antibody of the present disclosure (e.g., an anti-TREM2 antagonist antibody described herein), instructions for using the isolated antibody of the present disclosure in combination with an antibody and/or stimulatory cytokine, or instructions for using an isolated antibody of the present disclosure and an antibody and/or stimulatory cytokine, according to any methods of this disclosure.

[0389] The instructions generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the present disclosure are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

[0390] The label or package insert indicates that the composition is used for treating, e.g., a disease of the present disclosure. Instructions may be provided for practicing any of the methods described herein.

[0391] The kits of this disclosure are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an
intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (e.g., the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an isolated antibody of the present disclosure (e.g., an anti-TREM2 or anti-DAP12 antibody described herein). The container may further comprise a second pharmaceutically active agent.

[0392] Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

Diagnostic uses

[0393] The isolated antibodies of the present disclosure (e.g., an anti-TREM2 or anti-DAP12 antibody described herein) also have diagnostic utility. This disclosure therefore provides for methods of using the antibodies of this disclosure, or functional fragments thereof, for diagnostic purposes, such as the detection of TREM2 and/or DAP12 in an individual or in tissue samples derived from an individual.

[0394] In some embodiments, the individual is a human. In some embodiments, the individual is a human patient suffering from, or at risk for developing, cancer. In some embodiments, the diagnostic methods involve detecting TREM2 and/or DAP12 in a biological sample, such as a biopsy specimen, a tissue, or a cell. An isolated antibody of the present disclosure (e.g., an anti-TREM2 or anti-DAP12 antibody described herein) is contacted with the biological sample and antigen-bound antibody is detected. For example, a tumor sample (e.g., a biopsy specimen) may be stained with an anti-TREM2 or anti-DAP12 antibody described herein in order to detect and/or quantify tumor-associated macrophages (e.g., M2-type macrophages). The detection method may involve quantification of the antigen-bound antibody. Antibody detection in biological samples may occur with any method known in the art, including immunofluorescence microscopy, immunocytochemistry, immunohistochemistry, ELISA, FACS analysis, immunoprecipitation, or micro-positron emission tomography. In certain embodiments, the antibody is radiolabeled, for example with $^{18}$F and subsequently detected utilizing micro-
positron emission tomography analysis. Antibody-binding may also be quantified in a patient by non-invasive techniques such as positron emission tomography (PET), X-ray computed tomography, single-photon emission computed tomography (SPECT), computed tomography (CT), and computed axial tomography (CAT).

[0395] In other embodiments, an isolated antibody of the present disclosure (e.g., an anti-TREM2 or anti-DAP12 antibody described herein) may be used to detect and/or quantify, for example, microglia in a brain specimen taken from a preclinical disease model (e.g., a non-human disease model). As such, an isolated antibody of the present disclosure (e.g., an anti-TREM2 or anti-DAP12 antibody described herein) may be useful in evaluating therapeutic response after treatment in a model for a nervous system disease or injury such as dementia, frontotemporal dementia, Alzheimer’s disease, Nasu-Hakola disease, or multiple sclerosis, as compared to a control.

[0396] The invention will be more fully understood by reference to the following Examples. They should not, however, be construed as limiting the scope of the invention. All citations throughout the disclosure are hereby expressly incorporated by reference.

EXAMPLES

Example 1: Production, identification, and characterization of agonist anti-TREM2 antibodies

Introduction

[0397] The amino acid sequence of the human TREM2 preprotein is set forth below in SEQ ID NO: 1. Human TREM2 contains a signal peptide located at amino residues 1-18 of SEQ ID NO: 1. Human TREM2 contains an extracellular immunoglobulin-like variable-type (IgV) domain located at amino residues 29-112 of SEQ ID NO: 1; additional extracellular sequences located at amino residues 113-174 of SEQ ID NO: 1; a transmembrane domain located at amino residues 175-195 of SEQ ID NO: 1; and an intracellular domain located at amino residues 196-230 of SEQ ID NO: 1.
[0398]  TREM2 amino acid sequence (SEQ ID NO: 1):

```
10  20  30  40  50  60
MEPLRLILL PVTESGARN TTFFQGVAGQ SLQVSCPYDS MKHGVRRKAN CRQLEGEKPC
```

```
70  80  90  100  110  120
QRVYSTHNLW LLSFLRPNNG STAITDDTLG GTLTLNLRL QPHDAGLYQC QSLHSGEADT
```

```
130  140  150  160  170  180
LRKVLVEVLA DPLHRDAGD LWFPGESES F EDAHEHSIS RSLLEGLIFP PPTSILLLLA
```

```
190  200  210  220  230
CIFLIKILAA SALWAANHIG QKPQTHPSPS LDGOSHFDGYQ LQTLPLRLRT
```

[0399]  A known feature of human TREM2 is that the transmembrane domain contains a lysine (aa186) that can interact with an aspartic acid in DAP12, a key adaptor protein that transduces signaling from TREM2, TREM1, and other related IgV family members.

[0400]  A BLAST analysis of human TREM2 identified 18 related homologues. These homologues included the Natural Killer (NK) cell receptor NK-p44 (NCTR2), the polymeric immunoglobulin receptor (pIgR), CD300E, CD300A, CD300C, and TREML1/TLT1. The closest homologue was identified as NCTR2, having similarity with TREM2 within the IgV domain (FIG. 1A). A BLAST analysis also compared TREM proteins with other IgV family proteins (FIG. 1B).

[0401]  TREM2 is also related to TREM1. An alignment of the amino acid sequences of TREM1 and TREM2 was generated by 2-way blast (FIG. 2A). This is limited to the IgV domain as well.

[0402]  Antibodies that bind the extracellular domain of TREM2, particularly the extra cellular domain (amino acid residues 19-174 of SEQ ID NO: 1) are generated using mouse hybridoma technology, phage display technology, and yeast display technology. Antibodies are then screened for their ability to bind cells that express TREM2 and for their ability to activate TREM2 signaling and functions in cells and in a whole animal in vivo as described in Examples 2-31 below.

[0403]  For example, agonist anti-TREM2 antibodies can be produced that target the IgV domain (amino acid residues 29-112). IgV domains bind to targets, and through multimerization
of receptors, lead to activation. Thus these domains are rational targets for agonistic antibodies. They are also highly divergent.

[0404] Agonist anti-TREM2 antibodies can also be produced that target amino acid residues 99-174 of human TREM2. It is believed that amino acid residues 99-115 correspond to a peptide that blocks binding of TREM2 to its endogenous target, as the corresponding peptide in mouse TREM1 (amino acid residues 83-99) blocks binding of TREM1 to its endogenous target (Gibot et al., Infect. Immunity 2004). The mouse TREM1 peptide is called LP17 (LQVTDSGLYRCVIYHPP). The equivalent region in human TREM2 is located within the CD3 domain and is located at amino acid residues 99-115 of SEQ ID NO: 1 (LQPHDAGLYQCQSLHG). Antibodies that block ligand binding could activate the receptors similar to the ligand itself.

[0405] Another approach for predicting a relevant (e.g., agonistic) site within the human TREM2 protein is by targeting the sites where the mutations are found in Alzheimer’s disease (e.g., R47H), Polycystic lipomembranous osteodysplasia with sclerosing leuкоencephalopathy (PLOS1), or Nasu-Hakola disease. Also relevant is the site of the major mutations associated with human disease, which are generally found within the IgV domain.

[0406] The crystal structures of the TREM2-related structures of TREM1 (Kelker, MS et al., J Mol Biol, 2004. 344(5): p. 1175-81; Kelker, MS et al., J Mol Biol, 2004. 342(4): p. 1237-48; and Radaev, S et al., Structure, 2003. 11(12): p. 1527-35), TLT1 (Gattis, JL et al., J Biol Chem, 2006. 281(19): p. 13396-403), and NKP44 have been described, and thus structural regions/features are identified within the IgV domain that are particularly likely to play a central role in interacting with natural agonists. These studies support the belief that the complementary determining regions (CDR1, CDR2, CDR3) play a major role in ligand binding. TREM1 has been reported to be either monomeric (Gattis, JL et al., J Biol Chem, 2006. 281(19): p. 13396-403) or dimeric (Radaev, S et al., Structure, 2003. 11(12): p. 1527-35) in vitro under cell-free conditions, but its oligomeric state in vivo remains unclear, as well as that of TREM2.
The studies discussed herein describe the generation of agonist antibodies that bind TREM2. Antibodies were screened for binding to TREM2 expressing cells and for their ability to activate TREM2 signaling and functionality.

Results

Anti-TREM2 antibody production

Antibodies that bind the extracellular domain of TREM2, particularly within the extracellular sequences located at amino residues 113-174 of SEQ ID NO: 1, were generated using the following procedure. Eight naïve human synthetic yeast libraries each of ~10^9 diversity were designed, generated, and propagated as described previously (see, e.g., WO2009036379; WO2010105256; WO2012009568; Xu et al., (2013) Protein. Eng. Des. Sel. 26(10):663-670). The ADIMAB yeast-based antibody discovery platform used herein allowed for the identification of fully human, full-length, monoclonal IgG1 antibodies with broad epitopic coverage. The ADIMAB yeast is engineered to transport high quality, whole IgGs through the secretory pathway, and then present them on the surface or secrete them directly into the medium.

For the first rounds of selection, a magnetic bead sorting technique utilizing the Miltenyi MACs system was performed, as previously described (Siegel et al., (2004) J. Immunol. Methods 286(1-2):141-53). Briefly, yeast cells (~10^10 cells/library) were incubated with 3 ml of 200 nM biotinylated TREM2 antigen or 10 nM biotinylated TREM2-Fc fusion antigen for 15 min at room temperature in FACS wash buffer PBS with 0.1% BSA. Biotinylations were performed using the EZ-Link Sulfo-NHS-Biotinylation Kit (Thermo Scientific, Cat #21425). After washing once with 50 ml ice-cold wash buffer, the cell pellet was resuspended in 40 mL wash buffer, and 500 μl Streptavidin MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany. Cat # 130-048-101) were added to the yeast and incubated for 15 min at 4°C. Next, the yeast were pelleted, resuspended in 5 mL wash buffer, and loaded onto a MACS LS column (Miltenyi Biotec, Bergisch Gladbach, Germany. Cat.# 130-042-401). After the 5 mL was loaded, the column was washed 3 times with 3 mL FACS wash buffer. The column was then removed from the magnetic field, and the yeast were eluted with 5 mL of growth media and then grown overnight. The following three rounds of sorting were performed using flow cytometry. Approximately 1×10^8 yeast were pelleted, washed three times with wash buffer, and incubated
with 200 nM, 100, or 10 biotinylated TREM2 for 10 min at room temperature respectively. Yeast were then washed twice and stained with goat anti-human F(ab')2 kappa-FITC diluted 1:100 (Southern Biotech, Birmingham, Alabama, Cat# 2062-02) and either streptavidin-Alexa Fluor 633 (Life Technologies, Grand Island, NY, Cat # S21375) diluted 1:500 or Extravidin-phycoerythrin (Sigma-Aldrich, St Louis, Cat # E4011) diluted 1:50 secondary reagents for 15 min at 4°C. After washing twice with ice-cold wash buffer, the cell pellets were resuspended in 0.4 mL wash buffer and transferred to strainer-capped sort tubes. Sorting was performed using a FACS ARIA sorter (BD Biosciences) and sort gates were determined to select only TREM2 binding clones for two rounds and the third round was a negative sort to decrease reagent binders. After the final round of sorting, yeast were plated and individual colonies were picked for characterization.

[0410] Yeast clones were grown to saturation and then induced for 48 h at 30°C with shaking. After induction, yeast cells were pelleted and the supernatants were harvested for purification. IgGs were purified using a Protein A column and eluted with acetic acid, pH 2.0. Fab fragments were generated by papain digestion and purified over KappaSelect (GE Healthcare LifeSciences, Cat # 17-5458-01).

[0411] A total of 87 antibodies were generated. The antibodies were then screened for TREM2 binding. Antibodies that were positive for binding to primary cells were tested for agonistic activity. From the 87 antibodies, certain antibodies (e.g., Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65) were selected for further analysis.

**Antibody heavy chain and light chain variable domain sequences**

[0412] Using standard techniques, the amino acid sequences encoding the heavy chain variable (FIG. 2B) and the light chain variable (FIG. 2C) domains of the generated antibodies were determined. The Kabat CDR sequences of the antibodies are set forth in Table 1.
Table 1: Kabat CDR sequences

<table>
<thead>
<tr>
<th>Antibody Name</th>
<th>CDR H1</th>
<th>CDR H2</th>
<th>CDR H3</th>
<th>CDR L1</th>
<th>CDR L2</th>
<th>CDR L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab1</td>
<td>FTFSSYAMS</td>
<td>VIGSGGTYYADSVKG (SEQ ID NO:25)</td>
<td>AKGTPTLFQH</td>
<td>RASQVSSNLA (SEQ ID NO:120)</td>
<td>GASTRAT</td>
<td>QQLPYWPPT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:50)</td>
<td>(SEQ ID NO:130)</td>
<td>(SEQ ID NO:138)</td>
<td>(SEQ ID NO:153)</td>
</tr>
<tr>
<td>Ab2</td>
<td>FTSSSAMS</td>
<td>AIGSGGTYYADSVKG (SEQ ID NO:26)</td>
<td>AKVPSDYSYWGYSNYYNYMDV (SEQ ID NO:51)</td>
<td>RASQVGSNLA (SEQ ID NO:121)</td>
<td>GASTRAT</td>
<td>QYFYFYPPT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEQ ID NO:4)</td>
<td></td>
<td>(SEQ ID NO:138)</td>
<td></td>
<td>(SEQ ID NO:154)</td>
</tr>
<tr>
<td>Ab3</td>
<td>GTFSSYASTS</td>
<td>GIPFSTANYAQKFQG (SEQ ID NO:27)</td>
<td>AREQYHVMDV</td>
<td>QASQDISNYLN (SEQ ID NO:122)</td>
<td>DASNLAT</td>
<td>QQPFPYPYPT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEQ ID NO:5)</td>
<td></td>
<td>(SEQ ID NO:139)</td>
<td></td>
<td>(SEQ ID NO:155)</td>
</tr>
<tr>
<td>Ab4</td>
<td>GTFSSYASTS</td>
<td>GIPFSTASYAQKFQG (SEQ ID NO:28)</td>
<td>ARGVDSIMDY</td>
<td>RASQVSSSNLA (SEQ ID NO:120)</td>
<td>SASTRAT</td>
<td>QQDHDYPYPT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEQ ID NO:5)</td>
<td></td>
<td>(SEQ ID NO:140)</td>
<td></td>
<td>(SEQ ID NO:156)</td>
</tr>
<tr>
<td>Ab5</td>
<td>YFTSYYIH</td>
<td>IINPSSGTSYAQKFQG (SEQ ID NO:29)</td>
<td>ARAPQESPVFDI</td>
<td>RASQVSSSYL (SEQ ID NO:123)</td>
<td>GASSRAT</td>
<td>QYFSSSPFT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEQ ID NO:6)</td>
<td></td>
<td>(SEQ ID NO:141)</td>
<td></td>
<td>(SEQ ID NO:157)</td>
</tr>
<tr>
<td>Ab6</td>
<td>YFTSYYMH</td>
<td>IINPSSGTSYAQKFQG (SEQ ID NO:30)</td>
<td>ARGSPTYGYLEDP</td>
<td>RASQVSSSYLA (SEQ ID NO:124)</td>
<td>DASKRAT</td>
<td>QRVNLPTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEQ ID NO:7)</td>
<td></td>
<td>(SEQ ID NO:142)</td>
<td></td>
<td>(SEQ ID NO:158)</td>
</tr>
<tr>
<td>Ab7</td>
<td>YFTSYYMH</td>
<td>IINPSSGTSYAQKFQG (SEQ ID NO:31)</td>
<td>ARTSSKRERY</td>
<td>RASQVSSSYLA (SEQ ID NO:124)</td>
<td>DASKRAT</td>
<td>QRRSGSYPT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEQ ID NO:7)</td>
<td></td>
<td>(SEQ ID NO:142)</td>
<td></td>
<td>(SEQ ID NO:159)</td>
</tr>
<tr>
<td>Ab8</td>
<td>GSISSSSYWG</td>
<td>SISYSGTYYYNPSLKS (SEQ ID NO:32)</td>
<td>ARGPYRLLGLMDV</td>
<td>RASQSISSSYLN (SEQ ID NO:125)</td>
<td>GASSLQS</td>
<td>QQIDTDPT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEQ ID NO:8)</td>
<td></td>
<td>(SEQ ID NO:143)</td>
<td></td>
<td>(SEQ ID NO:160)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR H2</td>
<td>CDR H3</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Ab9</td>
<td>YSFTSYWIG</td>
<td>IYPGDSDTTYSIPS</td>
<td>ARLHISGEVNWFDP</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQFSYWPWT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:9)</td>
<td>(SEQ ID NO:33)</td>
<td>(SEQ ID NO:58)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:161)</td>
</tr>
<tr>
<td>Ab10</td>
<td>YSF1SNWIG</td>
<td>IYPGDSDTRYSIPS</td>
<td>AREGAODYGELAFDI</td>
<td>RASQSVSSSYLAA</td>
<td>GASSRAT</td>
<td>QHQHDSSPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:10)</td>
<td>(SEQ ID NO:34)</td>
<td>(SEQ ID NO:59)</td>
<td>(SEQ ID NO:123)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:162)</td>
</tr>
<tr>
<td>Ab11</td>
<td>YSF1TYWIG</td>
<td>IYPGDSDTRYSIPS</td>
<td>AREGHSDGGLGMDV</td>
<td>RASQSVSSDYLAA</td>
<td>GASSRAT</td>
<td>QQDYSYPWT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:11)</td>
<td>(SEQ ID NO:34)</td>
<td>(SEQ ID NO:60)</td>
<td>(SEQ ID NO:126)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:163)</td>
</tr>
<tr>
<td>Ab12</td>
<td>YSFTSYWIG</td>
<td>IYPGDSDTRYSIPS</td>
<td>ARLGHYSGTVSSYGM</td>
<td>RASQSISSYLN</td>
<td>AASSLQS</td>
<td>QQEYAVPYT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:9)</td>
<td>(SEQ ID NO:34)</td>
<td>(SEQ ID NO:61)</td>
<td>(SEQ ID NO:125)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:164)</td>
</tr>
<tr>
<td>Ab13</td>
<td>YTF1SYGTS</td>
<td>WISAYNGNTNYAQKLQG</td>
<td>ARGPSHYYDLA</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQVSNYPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:12)</td>
<td>(SEQ ID NO:35)</td>
<td>(SEQ ID NO:62)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:165)</td>
</tr>
<tr>
<td>Ab14</td>
<td>GSISSGGYYS</td>
<td>NYYSGSTVYNPSLKS</td>
<td>ARGLYGYGVLDV</td>
<td>QASQDISNYLN</td>
<td>DASNLET</td>
<td>QQVDDNPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:13)</td>
<td>(SEQ ID NO:36)</td>
<td>(SEQ ID NO:63)</td>
<td>(SEQ ID NO:122)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:166)</td>
</tr>
<tr>
<td>Ab15</td>
<td>GSISSGGYYS</td>
<td>NYYSGSTVYNPSLKS</td>
<td>ARGLYGYGVLDV</td>
<td>QASQDISNYLN</td>
<td>DASNLET</td>
<td>QQFDTTYPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:13)</td>
<td>(SEQ ID NO:36)</td>
<td>(SEQ ID NO:63)</td>
<td>(SEQ ID NO:122)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:167)</td>
</tr>
<tr>
<td>Ab16</td>
<td>GSISSNSYYWG</td>
<td>SIYSGSTTYNPSLKS</td>
<td>ARGVLGYGFVDY</td>
<td>QASQDISNYLN</td>
<td>DASNLET</td>
<td>QQFLNPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:14)</td>
<td>(SEQ ID NO:37)</td>
<td>(SEQ ID NO:64)</td>
<td>(SEQ ID NO:122)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:168)</td>
</tr>
<tr>
<td>Ab17</td>
<td>GSISSNSYYWG</td>
<td>SIYSGSTTYNPSLKS</td>
<td>ARGVLGYGFVDY</td>
<td>QASQDISNYLN</td>
<td>DASNLET</td>
<td>QQFFNFPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:14)</td>
<td>(SEQ ID NO:37)</td>
<td>(SEQ ID NO:64)</td>
<td>(SEQ ID NO:122)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:169)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR H2</td>
<td>CDR H3</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Ab18</td>
<td>GSIISSYYWS</td>
<td>SIYYSGSTNYNSL KS</td>
<td>ARDGGEYPGSGTP FDI</td>
<td>QASQDISNYLN</td>
<td>DASNLET</td>
<td>QQFIDLPLLFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:15)</td>
<td>(SEQ ID NO:38)</td>
<td>(SEQ ID NO:65)</td>
<td>(SEQ ID NO:122)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:170)</td>
</tr>
<tr>
<td>Ab19</td>
<td>GSIISSYYWS</td>
<td>SIYYSGSTNYNSL KS</td>
<td>ARDGGEYPGSGTP FDI</td>
<td>QASQDISNYLN</td>
<td>DASNLET</td>
<td>QQYYDPLLFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:15)</td>
<td>(SEQ ID NO:38)</td>
<td>(SEQ ID NO:65)</td>
<td>(SEQ ID NO:122)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:171)</td>
</tr>
<tr>
<td>Ab20</td>
<td>GSIISSYYWS</td>
<td>SIYYSGSTNYNSL KS</td>
<td>ARSGMASHFYDY</td>
<td>RASQSVSSSYL A</td>
<td>GASSRAT</td>
<td>QQFSSHPLLFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:15)</td>
<td>(SEQ ID NO:38)</td>
<td>(SEQ ID NO:66)</td>
<td>(SEQ ID NO:126)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:172)</td>
</tr>
<tr>
<td>Ab21</td>
<td>YSFTTYWG</td>
<td>IIYPGDSDTRYSPS FQG</td>
<td>ARAGHYDGHHGLG MDV</td>
<td>RASQSVSSSYL A</td>
<td>GASNRAT</td>
<td>QQDSDAPYT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:404)</td>
<td>(SEQ ID NO:405)</td>
<td>(SEQ ID NO:406)</td>
<td>(SEQ ID NO:407)</td>
<td>(SEQ ID NO:408)</td>
<td>(SEQ ID NO:409)</td>
</tr>
<tr>
<td>Ab22</td>
<td>YSFTTYWG</td>
<td>IIYPGDSDTRYSPS FQG</td>
<td>ARAGHYDGHHGLG MDV</td>
<td>RASQSVSSSYL A</td>
<td>GASSRAT</td>
<td>QQDDRSPYT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:11)</td>
<td>(SEQ ID NO:34)</td>
<td>(SEQ ID NO:60)</td>
<td>(SEQ ID NO:123)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:173)</td>
</tr>
<tr>
<td>Ab23</td>
<td>FITSSYAMS</td>
<td>AISGSGSTYAYAD SVKG</td>
<td>AGLGCHSMMDV</td>
<td>KSSQSLYSSN NKNYLA</td>
<td>WASTRES</td>
<td>QQAYLPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:67)</td>
<td>(SEQ ID NO:127)</td>
<td>(SEQ ID NO:147)</td>
<td>(SEQ ID NO:174)</td>
</tr>
<tr>
<td>Ab24</td>
<td>FITSSYAMS</td>
<td>AISGSGSTYAYAD SVKG</td>
<td>AKPLKRGFRFYP</td>
<td>RASQSISSYLN</td>
<td>AASSIQS</td>
<td>QQAFSPPPWT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:68)</td>
<td>(SEQ ID NO:125)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:175)</td>
</tr>
<tr>
<td>Ab25</td>
<td>FITSSYAMS</td>
<td>VISGSGSTYAYAD SVKG</td>
<td>AKEGRTTIMD</td>
<td>RASQSVSSSYL A</td>
<td>GASSRAT</td>
<td>QQDQRSPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:25)</td>
<td>(SEQ ID NO:69)</td>
<td>(SEQ ID NO:123)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:176)</td>
</tr>
<tr>
<td>Ab26</td>
<td>FITSSYAMS</td>
<td>VISGSGSTYAYAD SVKG</td>
<td>AKDQYSLDY</td>
<td>RASQSVSSYLA</td>
<td>DASNRT</td>
<td>QQEFIDLPLLFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:25)</td>
<td>(SEQ ID NO:70)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:177)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR H2</td>
<td>CDR H3</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Ab27</td>
<td>FITSSYAMS</td>
<td>AISGSGGSTYAD SVKG</td>
<td>AKKYSSRGVYFD Y</td>
<td>RASQS VSSYLA</td>
<td>DASN RAT</td>
<td>QQYNNFPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:71)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:178)</td>
</tr>
<tr>
<td>Ab28</td>
<td>FITSSYAMS</td>
<td>AISGSGGSTYAD SVKG</td>
<td>ARLGGAVGARIIV TYFDY</td>
<td>RASQS VSSYLA</td>
<td>DASKRAT</td>
<td>QRHYLRPTT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:72)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:142)</td>
<td>(SEQ ID NO:179)</td>
</tr>
<tr>
<td>Ab29</td>
<td>FITSSYGMH</td>
<td>VISYDGSNKYYAD SVKG</td>
<td>ARGQYYGGSGWF DP</td>
<td>RASQS VSSYLA</td>
<td>GASSRAT</td>
<td>QQPGAVPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:16)</td>
<td>(SEQ ID NO:39)</td>
<td>(SEQ ID NO:73)</td>
<td>(SEQ ID NO:123)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:180)</td>
</tr>
<tr>
<td>Ab30</td>
<td>FITSSYAMS</td>
<td>AISGSGGSTYAD SVKG</td>
<td>ARLGQYEAYFQH</td>
<td>RASQS ISSSYLN</td>
<td>GASSLQS</td>
<td>QQVYITPTT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:74)</td>
<td>(SEQ ID NO:125)</td>
<td>(SEQ ID NO:143)</td>
<td>(SEQ ID NO:181)</td>
</tr>
<tr>
<td>Ab31</td>
<td>FITSSYGMH</td>
<td>LIWYDGSNKYYA DSVKG</td>
<td>ARR RDGYYDEVF DI</td>
<td>QAS QDISNFLN</td>
<td>DASNLET</td>
<td>QQPVDLPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:16)</td>
<td>(SEQ ID NO:40)</td>
<td>(SEQ ID NO:75)</td>
<td>(SEQ ID NO:128)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:182)</td>
</tr>
<tr>
<td>Ab32</td>
<td>FITSSYAMS</td>
<td>AISGSGGSTYAD SVKG</td>
<td>ARVPKHYVVLDY</td>
<td>RASQS VSSYLA</td>
<td>DASN RAT</td>
<td>QQYSFFPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:76)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:183)</td>
</tr>
<tr>
<td>Ab33</td>
<td>FITSSYGMH</td>
<td>VISYDGSNKYYAD SVKG</td>
<td>ARAGGHLFDY</td>
<td>RASQS VSSYLA</td>
<td>DASN R AT</td>
<td>QQDSSFPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:16)</td>
<td>(SEQ ID NO:39)</td>
<td>(SEQ ID NO:77)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:184)</td>
</tr>
<tr>
<td>Ab34</td>
<td>FITSSYGMH</td>
<td>VISYDGSNKYYAD SVKG</td>
<td>ARDRGGEYVDFAF DI</td>
<td>RASQS ISSYLN</td>
<td>AASSQLQS</td>
<td>QQSFPPWT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:16)</td>
<td>(SEQ ID NO:39)</td>
<td>(SEQ ID NO:78)</td>
<td>(SEQ ID NO:125)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:185)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR H2</td>
<td>CDR H3</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Ab35</td>
<td>FITSSYAMS</td>
<td>AISGSGGSTYYAD SVKG</td>
<td>ARTSGYGASNYYF DY</td>
<td>RASQSISSYLN</td>
<td>AASSLQS</td>
<td>QQQGYSAPTT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:79)</td>
<td>(SEQ ID NO:125)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:186)</td>
</tr>
<tr>
<td>Ab36</td>
<td>FITSTYGMH</td>
<td>VIWYDGSNKYYA DSVKG</td>
<td>ARGTGAAASPAFDI</td>
<td>RASQSVSSY1.A</td>
<td>DASNRAT</td>
<td>QQIGDWPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:17)</td>
<td>(SEQ ID NO:41)</td>
<td>(SEQ ID NO:80)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:187)</td>
</tr>
<tr>
<td>Ab37</td>
<td>FITSSYAMS</td>
<td>AISGSGGSTYYAD SVKG</td>
<td>ARVGQYMLGMDDSIV</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQRAFLFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:81)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:188)</td>
</tr>
<tr>
<td>Ab38</td>
<td>FITSTYGMH</td>
<td>VIWYDGSNKYYA DSVKG</td>
<td>ARGAPVDYGGIPEYFQH</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQIDFLPYT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:17)</td>
<td>(SEQ ID NO:41)</td>
<td>(SEQ ID NO:82)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:189)</td>
</tr>
<tr>
<td>Ab39</td>
<td>FITSSYAMS</td>
<td>AISGSGGSTYYAD SVKG</td>
<td>AKHYHVGIADDI</td>
<td>RASQSISSYLN</td>
<td>AASSLQS</td>
<td>QQVYSPPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:83)</td>
<td>(SEQ ID NO:125)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:190)</td>
</tr>
<tr>
<td>Ab40</td>
<td>FITSSYAMS</td>
<td>AISGSGGSTYYAD SVKG</td>
<td>ARTSGYGASNYYFDY</td>
<td>RASQSISSYLN</td>
<td>AASSLQS</td>
<td>QQGYAAPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:3)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:79)</td>
<td>(SEQ ID NO:125)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:191)</td>
</tr>
<tr>
<td>Ab41</td>
<td>FITSTYAMS</td>
<td>AISGSGGSTYYAD SVKG</td>
<td>ARAMARKSVAFDI</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQRYALPTT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:18)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:84)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:192)</td>
</tr>
<tr>
<td>Ab42</td>
<td>FITSSSAMS</td>
<td>AISGSGGSTYYAD SVKG</td>
<td>AKVPSYQRTAFDP</td>
<td>RASQSVSSSYLAA</td>
<td>GASSRAT</td>
<td>QQYASPPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:4)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:85)</td>
<td>(SEQ ID NO:123)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:193)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR H2</td>
<td>CDR H3</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Ab43</td>
<td>FITSSAMS</td>
<td>AIGSGGSTYYADSVKG</td>
<td>AKSPAVAGIYRADY</td>
<td>RASQISRYLN</td>
<td>AASSLQS</td>
<td>QQVYSTPTT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:4)</td>
<td>(SEQ ID NO:26)</td>
<td>(SEQ ID NO:36)</td>
<td>(SEQ ID NO:129)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:194)</td>
</tr>
<tr>
<td>Ab44</td>
<td>FITSTYGMH</td>
<td>VIWYDGSKNYYADSVKGDVG</td>
<td>ARGTGAAASSPADI</td>
<td>RASQSVSSYLA</td>
<td>DSSNRAT</td>
<td>QQVLYWPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:17)</td>
<td>(SEQ ID NO:41)</td>
<td>(SEQ ID NO:80)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:148)</td>
<td>(SEQ ID NO:195)</td>
</tr>
<tr>
<td>Ab45</td>
<td>YTFTSYYMH</td>
<td>IINPSGGSSTYAQKFGQ</td>
<td>ARGPYTALDDYDYMDYV</td>
<td>RASQSVSSNLA</td>
<td>GASTRAT</td>
<td>QQVDDWFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:87)</td>
<td>(SEQ ID NO:120)</td>
<td>(SEQ ID NO:138)</td>
<td>(SEQ ID NO:196)</td>
</tr>
<tr>
<td>Ab46</td>
<td>YTFTSYYMH</td>
<td>IINPSGGSSTYAQKFGQ</td>
<td>ARPAKTADY</td>
<td>RASQSVSSYLA</td>
<td>DSSNRAT</td>
<td>QQRSNPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:88)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:148)</td>
<td>(SEQ ID NO:197)</td>
</tr>
<tr>
<td>Ab47</td>
<td>YTFTSYYMH</td>
<td>IINPSGGSSTYAQKFGQ</td>
<td>ARPGKSMDV</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QRRILYPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>(SEQ ID NO:31)</td>
<td>(SEQ ID NO:89)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:198)</td>
</tr>
<tr>
<td>Ab48</td>
<td>YTFTSYYMH</td>
<td>IINPSGGSSTYAQKFGQ</td>
<td>ARPGKSMDV</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQRAAYPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>(SEQ ID NO:31)</td>
<td>(SEQ ID NO:89)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:199)</td>
</tr>
<tr>
<td>Ab49</td>
<td>YTFTSYYMH</td>
<td>IINPSGGSSTYAQKFGQ</td>
<td>ARPAKTADY</td>
<td>RASQSVSSYLA</td>
<td>DASKRAT</td>
<td>QRTSHPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:88)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:142)</td>
<td>(SEQ ID NO:200)</td>
</tr>
<tr>
<td>Ab50</td>
<td>YTFTSYYIH</td>
<td>IINPSGGSSTYAQKFGQ</td>
<td>ARAPQESPYYVFDI</td>
<td>RASQSVSSSYLAW</td>
<td>GASSRAT</td>
<td>QQYAGSPFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:6)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:54)</td>
<td>(SEQ ID NO:123)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:201)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR H2</td>
<td>CDR H3</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Ab51</td>
<td>YTFTSYYMH</td>
<td>INPSGGSTSYAQK FQG</td>
<td>ARGVGGQDDYYYYM DV</td>
<td>RASQSISYSLN</td>
<td>AASSLQS</td>
<td>QQFDDVFET</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:90)</td>
<td>(SEQ ID NO:125)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:202)</td>
</tr>
<tr>
<td>Ab52</td>
<td>YTFTSYYIH</td>
<td>INPSGGSTSYAQK FQG</td>
<td>AREADSSGYPLGL DV</td>
<td>RASQSVISYNLA</td>
<td>GASTRAT</td>
<td>QQVNSLPFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:398)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:40)</td>
<td>(SEQ ID NO:401)</td>
<td>(SEQ ID NO:402)</td>
<td>(SEQ ID NO:403)</td>
</tr>
<tr>
<td>Ab53</td>
<td>YTFTSYYIH</td>
<td>INPSGGSTSYAQK FQG</td>
<td>ARAPQESPYVFDI</td>
<td>RASVSNSSYNLA</td>
<td>GASSRAT</td>
<td>QQVNSPFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:6)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:54)</td>
<td>(SEQ ID NO:123)</td>
<td>(SEQ ID NO:141)</td>
<td>(SEQ ID NO:203)</td>
</tr>
<tr>
<td>Ab54</td>
<td>YTFTSYYMH</td>
<td>INPSGGSTSYAQK FQG</td>
<td>ARGPGTIALDDYY YMDV</td>
<td>RASQSINSYLN</td>
<td>AASSLQS</td>
<td>QSSDDPPF</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:87)</td>
<td>(SEQ ID NO:130)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:204)</td>
</tr>
<tr>
<td>Ab55</td>
<td>YTFTGSYMH</td>
<td>WNPNSGGTNYA QKFQG</td>
<td>ARGPLYHMPMIFDYG</td>
<td>RASVSISYSLA</td>
<td>DASNRAT</td>
<td>QQLSTYPLT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:19)</td>
<td>(SEQ ID NO:42)</td>
<td>(SEQ ID NO:91)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:205)</td>
</tr>
<tr>
<td>Ab56</td>
<td>YTFTGYYMH</td>
<td>SINPNSGATNYA QKFQG</td>
<td>ARASSVDN</td>
<td>RASVSISYSLA</td>
<td>DASNRAT</td>
<td>QQRSSVYPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:20)</td>
<td>(SEQ ID NO:43)</td>
<td>(SEQ ID NO:92)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:206)</td>
</tr>
<tr>
<td>Ab57</td>
<td>YTFTNYGIS</td>
<td>WSAAYNGNTNYA QKLQG</td>
<td>ARGPTKAYGSGS YYVFDP</td>
<td>RASQSISYSLA</td>
<td>DASKRAT</td>
<td>QQVSLLPFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:21)</td>
<td>(SEQ ID NO:35)</td>
<td>(SEQ ID NO:93)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:142)</td>
<td>(SEQ ID NO:207)</td>
</tr>
<tr>
<td>Ab58</td>
<td>YSFTSYWIG</td>
<td>IYFGSDSTTRYPS FQG</td>
<td>ARLGIYSTGATAF DI</td>
<td>RASQSISWNL</td>
<td>DASSLES</td>
<td>LDYNYSPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:9)</td>
<td>(SEQ ID NO:34)</td>
<td>(SEQ ID NO:94)</td>
<td>(SEQ ID NO:131)</td>
<td>(SEQ ID NO:149)</td>
<td>(SEQ ID NO:208)</td>
</tr>
<tr>
<td>Ab59</td>
<td>YTFTGSYMH</td>
<td>WNPNSGGTNYA QKFQG</td>
<td>ARGGVWYSLFDI</td>
<td>QASQDISNYLN</td>
<td>DASNLET</td>
<td>QQHIALPFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:19)</td>
<td>(SEQ ID NO:42)</td>
<td>(SEQ ID NO:95)</td>
<td>(SEQ ID NO:122)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:209)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR H2</td>
<td>CDR H3</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Ab60</td>
<td>YTFTGYMMH</td>
<td>WINPNSGGTSYAQ</td>
<td>ARASKMGDD</td>
<td>RASQSVSSYLA</td>
<td>DASKRAT</td>
<td>QRASMPITT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:20)</td>
<td>KFQG (SEQ ID NO:44)</td>
<td>(SEQ ID NO:96)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:142)</td>
<td>(SEQ ID NO:210)</td>
</tr>
<tr>
<td>Ab61</td>
<td>YTFTSYGIIH</td>
<td>WISAAYNGTNYA</td>
<td>ARGGVPRVSYFQH</td>
<td>RASQSVSSYLA</td>
<td>DSSNRAT</td>
<td>QAFAFNRPPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:22)</td>
<td>QKQLQG (SEQ ID NO:35)</td>
<td>(SEQ ID NO:97)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:148)</td>
<td>(SEQ ID NO:211)</td>
</tr>
<tr>
<td>Ab62</td>
<td>YSFTSYWIG</td>
<td>IYPGDSDTRYSFPS</td>
<td>ARGZHDDWSGL</td>
<td>RASQSVSSYLA</td>
<td>DASKRAT</td>
<td>QQSSVHPYT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:9)</td>
<td>FQG (SEQ ID NO:34)</td>
<td>GLDV (SEQ ID NO:98)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:142)</td>
<td>(SEQ ID NO:212)</td>
</tr>
<tr>
<td>Ab63</td>
<td>YTFTSYGIS</td>
<td>WISTYNGTNYA</td>
<td>ARGSGSGYDSWYD</td>
<td>RASQGIDSWLA</td>
<td>AASSILQS</td>
<td>QQAYSLPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:12)</td>
<td>QKQLQG (SEQ ID NO:45)</td>
<td>D (SEQ ID NO:99)</td>
<td>(SEQ ID NO:132)</td>
<td>(SEQ ID NO:145)</td>
<td>(SEQ ID NO:213)</td>
</tr>
<tr>
<td>Ab64</td>
<td>YSFTSYWIG</td>
<td>IYPGDSDTRYSFPS</td>
<td>ARGZGRWSSGTAFAQ</td>
<td>RASQSVSSNLA</td>
<td>GASTRAT</td>
<td>QDDDDGTYT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:9)</td>
<td>FQG (SEQ ID NO:34)</td>
<td>DI (SEQ ID NO:100)</td>
<td>(SEQ ID NO:120)</td>
<td>(SEQ ID NO:138)</td>
<td>(SEQ ID NO:214)</td>
</tr>
<tr>
<td>Ab65</td>
<td>YSFTSYWIG</td>
<td>IYPGDSDTRYSFPS</td>
<td>ARGZGRKPSGSAVF</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QDYSWPUT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:9)</td>
<td>FQG (SEQ ID NO:34)</td>
<td>DI (SEQ ID NO:101)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:215)</td>
</tr>
<tr>
<td>Ab66</td>
<td>YTFTGSYMH</td>
<td>WINPNSGGTNYA</td>
<td>ARAGHKHTHYD</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QRSSAYPUT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:19)</td>
<td>QKFAQG (SEQ ID NO:42)</td>
<td>(SEQ ID NO:102)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:216)</td>
</tr>
<tr>
<td>Ab67</td>
<td>YTFTSYYMH</td>
<td>IIUPSGGTTYAQK</td>
<td>ARPZGKSMDV</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QRSSHFPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>FQG (SEQ ID NO:31)</td>
<td>(SEQ ID NO:89)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:217)</td>
</tr>
<tr>
<td>Ab68</td>
<td>FTFSYMGMH</td>
<td>LIWYDGNSKNYYAD</td>
<td>APKGSDMTDNY</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QRRANYPUT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:16)</td>
<td>DSVKG (SEQ ID NO:40)</td>
<td>(SEQ ID NO:103)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:218)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR H2</td>
<td>CDR H3</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Ab69</td>
<td>YTFTGSYM</td>
<td>WINPNSGGTNYA</td>
<td>ARAKSVDHHDY</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQRADYPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:19)</td>
<td>(SEQ ID NO:42)</td>
<td>(SEQ ID NO:104)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:219)</td>
</tr>
<tr>
<td>Ab70</td>
<td>YTFTGYYMH</td>
<td>WINPNSGGTSYAQ</td>
<td>ARASKMGDD</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQRSVVPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:20)</td>
<td>(SEQ ID NO:44)</td>
<td>(SEQ ID NO:96)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:206)</td>
</tr>
<tr>
<td>Ab71</td>
<td>YTFSSYMH</td>
<td>IINPSSGTTYA</td>
<td>ARDISTHDYDLAFDI</td>
<td>RASQSVSSYLA</td>
<td>GASNRAT</td>
<td>QQAGSHPFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:7)</td>
<td>(SEQ ID NO:29)</td>
<td>(SEQ ID NO:105)</td>
<td>(SEQ ID NO:123)</td>
<td>(SEQ ID NO:150)</td>
<td>(SEQ ID NO:220)</td>
</tr>
<tr>
<td>Ab72</td>
<td>GSISSYYWS</td>
<td>SIYSGSTYNPSLKS</td>
<td>ARSGTETFDY</td>
<td>QASQDITNYLN</td>
<td>DASNLET</td>
<td>QQDVNYPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:15)</td>
<td>(SEQ ID NO:38)</td>
<td>(SEQ ID NO:106)</td>
<td>(SEQ ID NO:133)</td>
<td>(SEQ ID NO:146)</td>
<td>(SEQ ID NO:221)</td>
</tr>
<tr>
<td>Ab73</td>
<td>YSI-TSYWG</td>
<td>VFGSDDTHTYPSFQG</td>
<td>ARAKMLDDGYAFDI</td>
<td>RASQSVSSSNL</td>
<td>GASTRAT</td>
<td>QQDDNYPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:9)</td>
<td>(SEQ ID NO:33)</td>
<td>(SEQ ID NO:107)</td>
<td>(SEQ ID NO:120)</td>
<td>(SEQ ID NO:138)</td>
<td>(SEQ ID NO:222)</td>
</tr>
<tr>
<td>Ab74</td>
<td>YTFTGSYMII</td>
<td>WINPNSGGTNYA</td>
<td>ARAHIKTDY</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQRSTFPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:19)</td>
<td>(SEQ ID NO:42)</td>
<td>(SEQ ID NO:102)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:223)</td>
</tr>
<tr>
<td>Ab75</td>
<td>YTFTGYYMH</td>
<td>WINPNSGGTNYA</td>
<td>ARDLGYSSLALD</td>
<td>RASQSVSSYLA</td>
<td>DASNRAT</td>
<td>QQVSNYPFT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:20)</td>
<td>(SEQ ID NO:42)</td>
<td>(SEQ ID NO:108)</td>
<td>(SEQ ID NO:124)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:224)</td>
</tr>
<tr>
<td>Ab76</td>
<td>FITSSYSMN</td>
<td>SIISSSSYYYADS</td>
<td>ARGGGRRGGDNNWFDP</td>
<td>KSSQSVLYSSN</td>
<td>WASTRES</td>
<td>QQYHDAPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:23)</td>
<td>(SEQ ID NO:46)</td>
<td>(SEQ ID NO:109)</td>
<td>(SEQ ID NO:127)</td>
<td>(SEQ ID NO:147)</td>
<td>(SEQ ID NO:225)</td>
</tr>
<tr>
<td>Ab77</td>
<td>FITSSYGGMH</td>
<td>VISYDGSKNYYADVKG</td>
<td>ARGPPHEMDY</td>
<td>KSSQSVLYSSN</td>
<td>WASTRES</td>
<td>QQAYVVPIT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:16)</td>
<td>(SEQ ID NO:39)</td>
<td>(SEQ ID NO:110)</td>
<td>(SEQ ID NO:127)</td>
<td>(SEQ ID NO:147)</td>
<td>(SEQ ID NO:226)</td>
</tr>
<tr>
<td>Antibody Name</td>
<td>CDR H1</td>
<td>CDR L1</td>
<td>CDR L2</td>
<td>CDR L3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A678</td>
<td>FITSSYMNH</td>
<td>YVYDGDSKRYYA</td>
<td>VQADNWPT</td>
<td>(SEQ ID NO:27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A679</td>
<td>FITSSYMNSN</td>
<td>YISGSSTIVYADSVK</td>
<td>DSNARAT</td>
<td>(SEQ ID NO:41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A680</td>
<td>GTHSYYAS</td>
<td>YYISHSGGYSVW</td>
<td>QQVNWPTT</td>
<td>(SEQ ID NO:228)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A681</td>
<td>GTHSYYAS</td>
<td>YMHSGGYSVW</td>
<td>QSSNWPTT</td>
<td>(SEQ ID NO:229)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A682</td>
<td>GSISGYYWS</td>
<td>YWSISGYSVW</td>
<td>QQYNWPTT</td>
<td>(SEQ ID NO:227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A683</td>
<td>GSISGYYWS</td>
<td>YWSISGYSVW</td>
<td>QQYNWPTT</td>
<td>(SEQ ID NO:227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A684</td>
<td>YTHISYGS</td>
<td>YWSISGYSVW</td>
<td>QQYNWPTT</td>
<td>(SEQ ID NO:227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A685</td>
<td>FITSSYMNH</td>
<td>YVYDGDSKRYYA</td>
<td>VKDGDDG</td>
<td>(SEQ ID NO:41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A686</td>
<td>FITSSYMNH</td>
<td>YVYDGDSKRYYA</td>
<td>VKDGDDG</td>
<td>(SEQ ID NO:41)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Attorney Docket No.: 735023000600

sf-3499172  - 179-
<table>
<thead>
<tr>
<th>Antibody Name</th>
<th>CDR H1</th>
<th>CDR H2</th>
<th>CDR H3</th>
<th>CDR L1</th>
<th>CDR L2</th>
<th>CDR L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab87</td>
<td>GSISSYYWS</td>
<td>SIYYSGSTNYNSLKS</td>
<td>ARHGWDVGWFD</td>
<td>RASQSVSRYLA</td>
<td>DASNRAT</td>
<td>QQYIFWPPT</td>
</tr>
<tr>
<td></td>
<td>(SEQ ID NO:15)</td>
<td>(SEQ ID NO:38)</td>
<td>(SEQ ID NO:119)</td>
<td>(SEQ ID NO:137)</td>
<td>(SEQ ID NO:144)</td>
<td>(SEQ ID NO:236)</td>
</tr>
</tbody>
</table>
Characterization of Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 binding

[0413] Initial characterization of TREM2 antibodies involved determining their ability to bind TREM2 expressed on dendritic and other primary human or mouse immune cells. Cells were harvested, plated at 10^5/ml in a 96 well plate, washed, and incubated in 100ul PBS containing 10-50 ug/ml Mab and Fc blocking reagent for 1 hour in ice. Cells were then washed twice and incubated in 100ul PBS containing 5ug/ml PE-conjugated secondary antibody for 30 minutes in ice. Cells were washed twice in cold PBS and acquired on a BD FACS Canto. Data analysis and calculation of MFI values was performed with FlowJo (TreeStar) software version 10.0.7.

[0414] Antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 demonstrated binding to a mouse cell line (BWZ T2) expressing recombinant mouse TREM2, as indicated by positive TREM2 antibody staining detected via FACS analysis (black outlined histograms) (FIG. 3A). The negative isotype control (antibody Ab88) did not demonstrate binding. Antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 demonstrated antibody binding to WT (Trem +/-) bone marrow derived mouse macrophages (BMMac, mMacs), but not to TREM2 deficient (TREM2 -/-) mouse macrophages (BMMac, mMacs) (FIG. 3B). Antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 demonstrated binding to both a human cell line (293) expressing recombinant Human TREM2 (FIG. 4A) and to primary human dendritic cells (hDC) (FIG. 4B). Conversely, antibodies Ab43 and Ab60 bound to a human cell line expressing recombinant human TREM2 (FIG. 4A), but did not bind to primary human dendritic cells (FIG. 4B).

[0415] Mean fluorescent intensities (MFI) values for cell types bound by TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 are listed in Table 4. Binding is compared to the parental mouse cell line (mTREM2 cell line BWZ parental), primary human cell line (hTREM2 Parental Cell line (293)), primary mouse macrophages deficient in TREM2 (mMacs KO MFI), and primary mouse dendritic cells deficient in TREM2 (mDC KO MFI). Results in Table 4 indicate that Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 bind specifically to cell lines overexpressing human and mouse TREM2 on the cell membrane, but not to control cell lines that do not express TREM2. The antibodies also bind to primary human macrophages and primary
mouse macrophages and dendritic cells. Binding to mouse primary cells is specific, as it is not detected on primary cells derived from TREM2 KO mice.

### Table 4: TREM2 Antibody Binding to Human and Mouse Cells

<table>
<thead>
<tr>
<th>Antibody</th>
<th>mTrem2 Cell line (BWZ-parental) MFI</th>
<th>mTrem2 Cell line (BWZ T2) MFI</th>
<th>hTrem2 Parental Cell line (293) MFI</th>
<th>hTrem2 Cell line (293) MFI</th>
<th>mMacs KO MFI</th>
<th>mMacs WT MFI</th>
<th>mDC KO MFI</th>
<th>mDC WT MFI</th>
<th>hDC % positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab1</td>
<td>960</td>
<td>12626</td>
<td>86</td>
<td>1491</td>
<td>69.8</td>
<td>82.3</td>
<td>235</td>
<td>393</td>
<td>64.9</td>
</tr>
<tr>
<td>Ab9</td>
<td>1044</td>
<td>15691</td>
<td>83</td>
<td>2126</td>
<td>97.2</td>
<td>171.0</td>
<td>200</td>
<td>538</td>
<td>65.8</td>
</tr>
<tr>
<td>Ab14</td>
<td>852</td>
<td>11550</td>
<td>87</td>
<td>1656</td>
<td>77.9</td>
<td>145.0</td>
<td>218</td>
<td>529</td>
<td>75.8</td>
</tr>
<tr>
<td>Ab22</td>
<td>828</td>
<td>12451</td>
<td>82</td>
<td>1837</td>
<td>67.2</td>
<td>110.0</td>
<td>191</td>
<td>451</td>
<td>76.9</td>
</tr>
<tr>
<td>Ab45</td>
<td>1022</td>
<td>16288</td>
<td>141</td>
<td>2058</td>
<td>86.2</td>
<td>141.0</td>
<td>277</td>
<td>652</td>
<td>78.8</td>
</tr>
<tr>
<td>Ab65</td>
<td>1354</td>
<td>16122</td>
<td>93</td>
<td>1734</td>
<td>92.5</td>
<td>165.0</td>
<td>260</td>
<td>642</td>
<td>76.9</td>
</tr>
</tbody>
</table>

[0416] The binding affinity of each anti-TREM2 antibody was determined by measuring their $K_D$ by ForteBio or MSD-SET. ForteBio affinity measurements were performed as previously described (Estep et al., 2013) *Mabs* 5(2):270-8). Briefly, ForteBio affinity measurements were performed by loading IgGs on-line onto AHQ sensors. Sensors were equilibrated off-line in assay buffer for 30 min and then monitored on-line for 60 seconds for baseline establishment. Sensors with loaded IgGs were exposed to 100 nM antigen for 5 minutes, then transferred to assay buffer for 5 min for off-rate measurement. Kinetics were analyzed using the 1:1 binding model.

[0417] Equilibrium affinity measurements were performed as previously described (Estep et al., 2013) *Mabs* 5(2):270-8). Solution equilibrium titrations (SET) were performed in PBS + 0.1% IgG-Free BSA (BSF) with antigen held constant at 50 pM and incubated with 3-to 5-fold serial dilutions of antibody starting at 10 nM. Antibodies (20 nM in PBS) were coated onto standard bind MSD-ECL plates overnight at 4°C or at room temperature for 30 min. Plates were then blocked for 30 min with shaking at 700 rpm, followed by three washes with wash buffer (PBSF + 0.05% Tween 20). SET samples were applied and incubated on the plates for 150s with shaking at 700 rpm followed by one wash. Antigen captured on a plate was detected with 250ng/mL sulfofitag-labeled streptavidin in PBSF by incubation on the plate for 3 min. The plates were washed three times with wash buffer and then read on the MSD Sector Imager 2400 instrument.
using 1x Read Buffer T with surfactant. The percent free antigen was plotted as a function of titrated antibody in Prism and fit to a quadratic equation to extract the $K_D$. To improve throughput, liquid handling robots were used throughout MSD-SET experiments, including SET sample preparation.

Table 5 lists values representing the binding affinity ($K_D$) of antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 to a human TREM2 Fc fusion protein (hTREM2-Fc), a human monomeric His tagged TREM2 protein (hTREM2-HIS), and a mouse TREM2 Fc fusion protein (mTREM2-Fc).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IgG $K_D$ hTREM2-Fc (M) Avid</th>
<th>IgG $K_D$ hTREM2-HIS (M) Monovalent</th>
<th>IgG $K_D$ mTREM2-Fc (M) Avid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab1</td>
<td>7.05E-10</td>
<td>6.67E-09</td>
<td>4.86E-09</td>
</tr>
<tr>
<td>Ab9</td>
<td>3.48E-10</td>
<td>6.32E-09</td>
<td>N.B.</td>
</tr>
<tr>
<td>Ab14</td>
<td>5.51E-10</td>
<td>3.19E-09</td>
<td>6.20E-10</td>
</tr>
<tr>
<td>Ab22</td>
<td>3.06E-10</td>
<td>1.01E-09</td>
<td>3.40E-10</td>
</tr>
<tr>
<td>Ab45</td>
<td>2.29E-10</td>
<td>6.54E-10</td>
<td>N.B.</td>
</tr>
<tr>
<td>Ab65</td>
<td>5.46E-10</td>
<td>5.01E-09</td>
<td>1.56E-09</td>
</tr>
</tbody>
</table>

Example 2: Enhancement of the ability of BMDCs to induce antigen-specific T-cell proliferation by agonistic and/or bispecific TREM2 antibodies

It is believed that agonistic anti-TREM2, and/or TREM2 bispecific antibodies may increase ability of bone marrow-derived dendritic cells (BMDC) to express the markers CD83 and CD86 and then to induce antigen-specific T-cell proliferation. To determine if TREM2 antibodies induce expression of the cell surface markers CD83 and CD86 on dendritic cells, antibodies are plated overnight at 4°C in 12 well plates at 2 or 5 µg/ml in PBS. Wells are washed 3X with PBS the next day and day 5 immature human DCs are harvested and plated at 1 million cells per well and incubated at 37°C, 5% CO₂ in the absence of cytokine. FACS analysis of CD86, CD83 and CD11c (BD Biosciences) is performed on a BD FACS Canto 48 hours later. Data analysis was performed with FlowJo (TreeStar) software version 10.0.7. Alternatively, day 5 immature human dendritic cells are plated at 100,000 cells per well in a U-bottom non-TC treated 96 well plate in media without cytokine. Antibodies are added at 5 µg/ml with or without LPS-removed anti-human secondary antibody (Jackson ImmunoResearch) at 20 µg/ml. FACS
analysis for CD86, CD83, and CD11c (BD Biosciences) is performed 48hrs post antibody addition as previously described.

[0420] Ovalbumin (OVA)-specific T-cell response induced by BMDCs can be determined by CFSE dilution. BMDCs are isolated by MACS after 6 days of culture and plated at 1 \( \times 10^4 \) cells per well of a round bottom 96 well plate with OVA (2 or 0.5 mg/mL) and CpG DNA (100 or 25 nM) in the presence of GM-CSF (10 ng/mL) for 4 h. CD4 T-cells from the spleen and lymph nodes of OT-II transgenic mice are isolated by using Dynal Mouse CD4 Negative Isolation Kit (Invitrogen) and stained with CFSE (final 0.8 mM). After 4 h of DC culture, 1\( \times \)\( 10^5 \) CFSE-labeled CD4 OT-II T-cells are added into each well and incubated for 72 h. After culturing, cells are stained with an anti-CD4 monoclonal antibody and flow cytometry is performed to detect CFSE dilution of gated CD4 OT-II T-cells. Data analysis to calculate the percentage of divided and division index is performed by Flowjo software (Treestar) (Eur. J. Immunol. 2012. 42: 176–185).

[0421] Alternatively, Day 5 immature dendritic cells (CD14<sup>CD11c<sup>+</sup> LIN<sup>-</sup>) are plated in 12 well dishes coated the previous day with 2 \( \mu \)g/ml antibody. Plates are washed 3 times with PBS before addition of T cells. CD4<sup>+</sup> T cells from nonautologous donors were isolated and labeled with CFSE before addition to DCs in ratio of 1:10. CD3/CD28 Dynal beads serve as a positive control. Day 5 post co-culture cells are analyzed by flow cytometry on a BD FACSCanto II for CFSE dilution. Percent CFSE<sub>hi</sub> compared to CFSE<sub>lo</sub> cells are calculated for each condition with FlowJo (TreeStar).

Example 3: TREM2 antibodies induce the expression of CD83 and CD86 on human dendritic cells (DCs) and induce T cell proliferation

[0422] To evaluate the ability of anti-TREM2 antibodies to modify expression of CD83 and CD86, both plate bound and soluble antibodies were incubated with dendritic cells (DCs), and the expression of CD83, CD86, CCR7, and phosphorylated ERK were measured. To evaluate the ability of anti-TREM2 antibodies to modulate T cell proliferation, DCs were incubated with T cells and anti-TREM2 antibodies, and the level of T cell proliferation was measured.
Antibodies were plated overnight at 4C in 12 well plates at 2 or 5ug/ml in PBS. Wells were washed 3X with PBS the next day. On day 5, immature human DCs were harvested and plated at 1 million cells per well and incubated at 37C, 5% CO2 in the absence of cytokine. FACS analysis of CD86, CD83, CD11c, HLA-DR, and LIN (BD Biosciences) was performed on a BD FACS Canto 48 hours later. Data analysis was performed with FlowJo (TreeStar) software version 10.0.7. Levels of CD83, CD86, and CCR7 were evaluated for CD11c+HLA-DR+LIN-cell populations. For intracellular ERK phosphorylation, cells were fixed with 1% formaldehyde, permeabilized with cytofix/cytoperm kit (BD), and intracellular Erk phosphorylation was determined with flow cytometry after staining with PE-ERK antibody (BD).

Alternatively, Day 5 immature human dendritic cells were plated at 100,000 cells per well in a U-bottom non-TC treated 96 well plate in media without cytokine. Antibodies were added at 5ug/ml with or without LPS-removed anti-human secondary (Jackson ImmunoResearch) at 20ug/ml. FACS analysis for CD86, CD83, CD11c, HLA-DR, and LIN (BD Biosciences) was performed 48hrs post antibody addition as previously described.

Additionally, Day 5 immature dendritic cells (CD14CD11c+LIN-) were plated in 12 well dishes coated the previous day with 2ug/ml antibody. Plates were washed 3 times with PBS before addition of T cells. CD4+ T cells from non-autologous donors were isolated and labeled with CFSE before addition to DCs in ratio of 1:10, 1:50, or 1:250. CD3/CD28 Dynal beads serve as a positive control. Day 5 post co-culture cells were analyzed by flow cytometry on a BD FACSCanto II for CFSE dilution. Percent CFSEhi compared to CFSElo cells were calculated for each condition with FlowJo (TreeStar).

Plate bound TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 increased the frequency of CD83+CD86+ DCs compared to the isotype control antibody Ab88 (FIG. 5A). Soluble antibodies Ab1, Ab9, Ab14, Ab45, and Ab65, when cross-linked with anti-human secondary antibody, induced expression of CD86 on DCs. Conversely, cross-linked antibody Ab22 and non-cross-linked soluble antibodies did not induce CD86 expression (FIG. 5B). Based on these results, TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 function as
agonists to induce the expression of inflammatory surface markers CD83 and CD86 on human dendritic cells.

Example 4: Normalization and reduction of Toll-like receptor (TLR) responses in macrophages by agonistic and/or bispecific TREM2, antibodies

[0427] Bone marrow-derived macrophages (BMDM) or primary peritoneal macrophage responses are altered to TLR signaling by deficiency of TREM2 (Turnbull, IR et al., J Immunol 2006; 177:3520-3524). It is believed that agonistic anti-TREM2, and/or TREM2 bispecific antibodies may reduce and normalize TLR responses in macrophages.

[0428] To elicit primary macrophages, mice are treated with 1.5 ml of 2% thioglycollate medium by intraperitoneal injection, and cells are then isolated by peritoneal lavage. To generate BMDM, total bone marrow is cultured in DMEM supplemented with 10% bovine calf serum, 5% horse serum, and 6 ng/ml recombinant human CSF-1 (R&D Systems). Cells are cultured for 5-6 days, and adherent cells are detached with 1m MEDTA in PBS. Cells are stained with commercially available antibodies: anti-CD11b, anti-CD40, anti-GR1 (BD Pharmingen), and F4/80 (Caltag Laboratories).

[0429] BMDM are re-plated and allowed to adhere for 4 h at 37°C, and then TLR agonists, such as LPS (Salmonella abortus equi), zymosan (Saccharomyces cerevisiae), and CpG 1826 DNA (purchased from e.g., Sigma-Aldrich) are added. Cell culture supernatant is collected 24 h after stimulation and the levels of IFN-a4, IFN-b, IL-6, IL-12 p70, and TNF Cytokine concentrations in the culture supernatants are determined using mouse IFN-a4, IFN-b, IL-6, IL-12 p70, TNF, and IL-10 ELISA kits (eBioscience) and VeriKine Mouse IFN-b ELISA kit (PBL interferon source) according to manufacturer’s protocol. Alternatively Cytometric Bead Array for human or mouse cytokines (BD Biosciences), or a V-PLEX Human or mouse Cytokine system with the Meso scale discovery System can be used.

[0430] Alternatively, to analyze cytokines secretion BM derived macrophages of the indicated genotype are harvested at day 5 and plated on 96-well plate at 10^5 cells/well. Cells are then stimulated with the indicated concentration of LPS or zymosan. 24hours later, cell culture supernatants are harvested and analyzed by FACS for the presence of inflammatory cytokines
(IL-12, IL-10, IFN-γ, TNFa, IL-6, MCP-1) using a Cytometric Bead Array kit (BD, following manufacturer’s instructions). Cells are also analyzed by FACS to assess viability (DAPI) and expression of surface markers (CD11b, CD86).

Example 5: TREM2 decreases the secretion of inflammatory cytokines from mouse macrophages.

[0431] Bone marrow-derived macrophages (BMDM) or primary peritoneal macrophage responses possess altered TLR signaling when deficient in TREM2 (Turnbull, IR et al., J Immunol 2006; 177:3520-3524). In order to determine the role of TREM2 in inflammatory cytokine production, mouse wild-type (WT), TREM2 knock-out (KO), and TREM2 heterozygous (Het) macrophages were cultured with various inflammatory mediators, and cytokine levels were measured in the culture supernatants.

[0432] To generate BMDM, total bone marrow from wild-type (WT), TREM2 KO (KO), and TREM2 heterozygous (Het) mice was cultured in RPMI supplemented with 10% bovine calf serum, 5% horse serum, and 50 ng/ml recombinant mouse CSF-1 (R&D Systems). Cells were cultured for 5 days, and adherent cells were detached with 1mM EDTA in PBS. BMDM were plated on 96-well plates at 10^5 cells/well and allowed to adhere for 4 h at 37°C. Cells were then stimulated with TLR agonists LPS (Salmonella abortus equi) or zymosan (Saccharomyces cerevisiae) at concentrations ranging from 0.01-100ng/ml (LPS) or 0.01-100μg/ml (zymosan). Alternatively, macrophages isolated from WT, KO, and Het mice were cultured in the presence of 10ng/ml of the cytokine IL-4 or 50ng/ml of IFN-γ. Cell culture supernatant was collected 24 or 48 h after stimulation and the levels of TNFa, IL-6, IL-10, and MCP-1 cytokines were measured by using Cytometric Bead Array Mouse Inflammation Kit (BD) according to manufacturer’s protocol.

[0433] Wild-type (WT) macrophages stimulated with the inflammatory mediators LPS or zymosan secreted less inflammatory cytokines TNFa, IL-6, IL-10, and MCP-1 compared to TREM2 KO (TREM2^{−/−}) macrophages (FIG. 6A). Similarly, WT and Het (TREM2^{+/−}) macrophages treated with the mediator IFN-γ produced less inflammatory cytokines IL-6 and TNFa compared to TREM2 KO macrophages (FIG. 6B). WT, Het, and KO macrophages
cultured in the presence of the cytokine IL-4 produced similar low levels of IL-6 and TNFa (FIG. 6B). Based on these results, TREM2 antibodies may be able to reduce the secretion of inflammatory cytokines from macrophages.

Example 6: Induction of the anti-inflammatory cytokine IL-10 in bone marrow-derived myeloid precursor cells by agonistic and/or bispecific TREM2 antibodies

[0434] It is believed that bone marrow-derived myeloid precursor cells may show an increase in the anti-inflammatory cytokine IL-10 following treatment with agonistic anti-TREM2, and/or TREM2 bispecific antibodies and stimulation with 100 ng/ml LPS (Sigma), by co-culturing with apoptotic cells, or by a similar stimulus.

[0435] Isolation of bone marrow-derived myeloid precursor cells is performed as follows. Bone marrow cells are isolated from adult 6-8 week-old female C57BL/6 mice (Charles River, Sulzfeld, Germany) and from TREM2 deficient mice (KOMP repository) from the medullary cavities of the tibia and femur of the hind limbs. Removal of erythrocytes is performed by lysis with hypotonic solution. Cells are cultured in DMEM medium (Invitrogen) containing 10% fetal calf serum (Pan Biotech) and 10 ng/ml of GM-CSF (R&D Systems) in 75 cm² culture flasks (Greiner Bio-One). After 24 h, non-adherent cells are collected and re-seeded in fresh 75 cm² culture flasks. Medium is changed after 5 d and cells are cultured for an additional 10-11 d. Supernatant is collected after 24 h, and the level of IL-10 released from the cells is determined by IL-10 ELISA according to manufacturer’s instructions (QuintikineM mouse IL-10, R&D Systems) (JEM (2005), 201; 647–657; and PLoS Medicine (2004), 4 | Issue 4 | e124).

Example 7: Induction of phagocytosis in cells from the myeloid lineage by agonistic and/or bispecific TREM2 antibodies

[0436] It is believed that agonistic anti-TREM2 and/or TREM2 bispecific antibodies may induce phagocytosis of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, and disease-causing proteins, such as A beta peptide, alpha synuclein protein, Tau protein, TDP-43 protein, prion protein, huntingtin protein, RAN, translation products antigen, including the DiPeptide Repeats,(DPRs peptides) composed of glycine-alanine (GA), glycine-proline (GP), glycine-arginine (GR), proline-alanine (PA), or proline-
arginine (PR) in cells from the myeloid lineage, such as monocytes, Dendritic cells macrophages and microglia. The bispecific antibodies may be antibodies that recognize the TREM2 antigen and a second antigen that includes, without limitation, a beta peptide, antigen or an alpha synuclein protein antigen or, Tau protein antigen or, TDP-43 protein antigen or, prion protein antigen or, huntingtin protein antigen, or RAN, translation Products antigen, including the DiPeptide Repeats,(DPRs peptides) composed of glycine-alanine (GA), glycine-proline (GP), glycine-arginine (GR), proline-alanine (PA), or proline-arginine (PR).

[0437] Monocytes are isolated from peripheral blood that is collected from adult C57BL/6 mice. Hypotonic lysis buffer depletes erythrocytes. Cells are plated on culture dishes in RPMI medium (Invitrogen) containing 10% fetal calf serum (Pan Biotech). Cells are cultured for several hours at 37°C in 10% CO₂. After trypsinization, adherent cells are collected and used for phagocytosis experiments.

[0438] Microglial cells are prepared from the brains of post-natal day 3 to 5 (P3 to P5) C57BL/6 mice. In brief, meninges are removed mechanically, and the cells are dissociated by trituration and cultured in basal medium (BME; GIBCO BRL) supplemented with 10% FCS (PAN Biotech GmbH), 1% glucose (Sigma-Aldrich), 1% L-glutamine (GIBCO BRL), and 1% penicillin/streptomycin (GIBCO BRL), for 14 d to form a confluent glial monolayer. To collect microglial cells, the cultures are shaken on a rotary shaker (200 rpm) for 2 h. The attached astrocytes are used for immunohistochemistry. The detached microglial cells are seeded in normal culture dishes for 1 h, and then all non-adherent cells are removed and discarded. Purity of the isolated microglial cells is about 95% as determined by flow cytometry analysis with antibody directed against CD11b (BD Biosciences). Microglial cells are cultured in basal medium as previously described (Hickman SE et al., J Neurosci. 2008 Aug 13;28(33):8354-60; and Microglia Methods and Protocols Vol. 1041).

[0439] Oligodendrocytes (i.e., neurons) and neuron-enriched cells are prepared from the brain of C57BL/6 mouse embryos (E15-16). In brief, brain tissue is isolated and mechanically dispersed and seeded in culture dishes pre-coated with 0.01 mg/ml poly-L-ornithin (Sigma-Aldrich) and 10 μg/ml laminin (Sigma-Aldrich). Cells are cultured in neuronal condition medium
(BME; GIBCO BRL) supplemented with 2% B-27 supplement (GIBCO BRL), 1% glucose (Sigma-Aldrich), and 1% FCS (PAN Biotech GmbH). Cells are cultured for 5-10 d to obtain morphologically mature oligodendrocytes.

[0440] To conduct phagocytosis assays microglia, macrophages or dendritic cells are cultured with apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, and disease-causing proteins. Neurons are cultured for 5-10 d, and okadaic acid is then added at the final concentration of 30 nM for 3 h to induce apoptosis. Neuronal cell membranes are labeled with CellTracker CM-Dil membrane dye (Molecular Probes). After incubation, apoptotic neurons or other targets of phagocytosis are washed two times and added to the transduced microglial culture at an effector/target ratio of 1:20. At 1 and 24 h after addition of apoptotic neurons, the number of microglia having phagocytosed neuronal cell membranes is counted under a confocal fluorescence microscope (Leica). Apoptotic cells are counted in three different areas at a magnification of 60. The amount of phagocytosis is confirmed by flow cytometry. Moreover, 24, 48, or 72 h after the addition of apoptotic neurons, cells are collected and used for RT-PCR of cytokines.

[0441] To conduct microsphere bead or bacterial phagocytosis assay, microglia, macrophages or dendritic cells are treated with anti-TREM2 agonistic antibodies. After 24 h, 1.00 μm of red fluorescent microsphere beads (Fluoresbrite Polychromatic Red Mi-crospheres; Polysciences Inc.) or, fluorescent labeled bacteria are added for 1 h. Phagocytosis of microsphere beads or, fluorescent labeled bacteria, by microglia is analyzed by fluorescence microscopy. Furthermore, microglia are collected from the culture plates and analyzed by flow cytometry. The percentage of microglia having phagocytosed beads is determined. Because phagocytosis varies from one experiment to the other, the relative change in phagocytosis is also determined. Data are shown as the relative change in phagocytosis between microglia cultured with agonistic antibodies and control antibody.

[0442] To conduct RT-PCR for analysis of inflammatory gene transcripts, microglia are transduced with a TREM2 vector or a GFP1 control vector. Cells are then cultured on dishes and treated with anti-TREM2 agonistic antibodies. After 24, 48, and 72 h, RNA is isolated from
microglia using an RNeasy Mini Kit (QIAGEN). RNA is also collected from microglia that have been transduced with sh-TREM2 RNA, sh-control RNA, wtTREM2, GFP2, mtDAP12-GFP, and GFP1 vector and co-cultured with apoptotic neurons for 48 h.

Reverse transcription of RNA is then performed. Quantitative RT-PCR by SYBR Green is performed on an ABI Prism 5700 Sequence Detection System (PerkinElmer). Amplification of GAPDH is used for sample normalization. The amplification protocol followed the GeneAmp 5700 Sequence Detection System Software (version 1.3). For detection of GAPDH, TNF-alpha, IL-1, NOS2, and TGF-beta transcripts, the following forward and reverse primers were used at final concentrations of 200 nM:

GAPDH forward primer: 5’-CTCCACTCAGCGCAATTCAA-3’, and GAPDH reverse primer: 5’-GTGACAAGCTTCCCCATTCTCG-3’;

TNF-α forward primer: 5’-CGTCAGCTGATTTATCTCT-3’, and TNF-α reverse primer: 5’-ACGGCAGAGGAAGGTGACTT-3’;

IL-1α forward primer: 5’-ACAAGCCTCCTGCTG-3’, and IL-1α reverse primer: 5’-CCATTGAGGTGAGAGCTTCA-3’;

NOS2 forward primer: 5’-GGAACACCCAGCTACGTCTTC-3’, NOS2 reverse primer: 5’-TACCTCATTGCGCCAGCTT-3’; and

TGF-β1 forward primer: 5’-AGGACCTGGGTTGAAGTGG-3’, and TGF-β1 reverse primer: 5’-AGTTGGGATGGGTACCGCCTTG-3’.

To conduct amyloid phagocytosis assay, HiLyteFluor™ 647 (Anaspec)-Abeta-(1–40) is resuspended in Tris/EDTA (pH 8.2) at 20 mM and then incubated in the dark for 3 d at 37°C to promote aggregation. Microglial, macrophages or dendritic cells are pretreated in low serum (0.5% FBS supplemented with insulin), LPS (50 ng/ml), IFNc (100 units/ml), and anti-TREM2 agonistic antibodies for 24 h prior to the addition of aggregated fluorescently labeled a beta peptide. Amyloid phagocytosis and surface expression of TREM2 are determined by flow cytometric analysis 5 h post-addition of 100 nM aggregated HiLyteFluor™ 647–Ab-(1–40)
(ASN NEURO (2010) 2(3): 157-170). Phagocytosis of other disease-causing proteins is conducted in a similar manner.

Example 8: Induction of SYK and/or ERK activation by agonistic TREM2 and/or TEM2 bispecific antibodies

[0445] It is believed that agonistic anti-TREM2 and/or TREM2/ bispecific antibodies may induce Syk and ERK activation.

[0446] Microglia, macrophages or dendritic cells are exposed to agonistic anti-TREM2, and/or TREM2 bispecific antibodies for 1 h. After stimulation, cells are lysed in reducing sample buffer for Western blot analysis. Phosphorylation of ERK and total amount of Syk and/or ERK are determined by immuno-detection with anti-phospho-Syk or ERK and anti-Syk or ERK antibodies, respectively (both from Cell Signaling Technology) by Western blot analysis (JEM (2005), 201, 647–657).

Example 9: TREM2 antibodies Ab1, Ab9, Ab14, Ab20, Ab22, Ab45, and Ab65 induce Syk phosphorylation

[0447] Spleen tyrosine kinase (Syk) is an intracellular signaling molecule that functions downstream of TREM2 by phosphorylating several substrates, thereby facilitating the formation of a signaling complex leading to cellular activation and inflammatory processes. The ability of agonist TREM2 antibodies to induce Syk activation was determined by culturing human and mouse macrophages and primary human dendritic cells and measuring the phosphorylation state of Syk protein in cell extracts.

[0448] Bone marrow-derived macrophages (BMDM), WT mouse BMDM, TREM2 knockout (KO) mouse BMDM, and primary human dendritic cells were starved for 4 hours in 1% serum RPMI and then removed from tissue culture dishes with PBS-EDTA, washed with PBS, and counted. The cells were coated with full-length agonist TREM2 antibodies Ab1, Ab9, Ab14, Ab20, Ab22, Ab45, Ab65, non-agonistic antibodies (Ab16, Ab77), or control antibodies (Ab89, or Ab92) for 15 minutes on ice. After washing with cold PBS, cells were incubated at 37°C for the indicated period of time in the presence of goat anti-human IgG. After stimulation, cells were lysed with lysis buffer (1% v/v NP-40%, 50 Mm Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM
EDTA, 1.5 mM MgCl₂, 10% glycerol, plus protease and phosphatase inhibitors) followed by centrifugation at 16,000 g for 10 min at 4°C to remove insoluble materials. Lysates were then immunoprecipitated with anti-Syk Ab (N-19 for BMDM or 4D10 for human DCs, Santa Cruz Biotechnology). Precipitated proteins were fractionated by SDS-PAGE, transferred to PVDF membranes and probed with anti-phosphotyrosine Ab (4G10, Millipore). To confirm that all substrates were adequately immunoprecipitated, immunoblots were reprobed with anti-Syk Ab (Abcam, for BMDM) or anti-Syk (Novus Biological, for human DCs). Visualization was performed with the enhanced chemiluminescence (ECL) system (GE healthcare), as described (e.g., Peng et al., 2010 Sci Signal., 3(122): ra38).

TREM2 antibodies Ab1, Ab9, Ab14, Ab20, Ab22, Ab45, and Ab65 induced TREM-2 mediated Syk phosphorylation in BMDMs, (FIG. 7A). Syk phosphorylation induced by Antibodies Ab1, Ab9, and Ab45 is TREM-2 specific, as Syk phosphorylation is not induced when TREM2 KO BMDM are used as a control (FIG. 7B). TREM2 antibodies Ab22, Ab45, and Ab65 induce Syk phosphorylation in primary human dendritic cells (FIG. 7C). Non agonistic antibodies (Ab16 and Ab77) or control antibodies (Ab89 and Ab92) did not induce Syk phosphorylation. Based on these results, TREM2 antibodies Ab1, Ab9, Ab14, Ab20, Ab22, Ab45, Ab65 function as agonists to induce Syk phosphorylation in macrophages and dendritic cells.

Example 10: Induction of CCR7 and migration toward CCL19 and CCL21 in microglia, macrophages, and dendritic cells by agonistic TREM2, or TEM2 bispecific antibodies

It is believed that anti-TREM2, and/or TREM2/ bispecific antibodies may induce CCR7 and migration toward CCL19 and CCL21 in microglial cells, macrophages, and dendritic cells.

Microglial, macrophages or dendritic cells are either cultured with agonistic anti-TREM2, and/or TREM2/DAP12 bispecific antibodies, or with a control antibody. Cells are collected after 72 h, immuno-labeled with CCR7 specific anti-bodies, and analyzed by flow cytometry.
To determine any functional consequences of increased CCR7 expression, a chemotaxis assay is performed. Microglia, macrophages or dendritic cells are stimulated via TREM2 with the agonistic anti-TREM2, and/or TREM2/DAP12 bispecific antibodies and placed in a two-chamber system. The number of microglial cells migrating toward the chemokine ligands CCL19 and CCL21 is quantified (JEM (2005), 201, 647–657).

For the chemotaxis assay, microglial, macrophages or dendritic cells are exposed to the agonistic anti-TREM2 or TREM2/ bispecific antibodies and treated with 1 µg/ml LPS. Microglia, macrophages or dendritic cells are transferred into the upper chamber of a transwell system (3 µm pore filter; Millipore) containing 450 µl medium with 100 ng/ml CCL19 or CCL21 (both from PeproTech) in the lower chamber. After a 1 h incubation period, the number of microglial macrophages or dendritic cells that have migrated to the lower chamber is counted in three independent areas by microscopy (JEM (2005), 201, 647–657).

Example 11: Induction of F-actin in microglia, macrophages, and dendritic cells by agonistic TREM2, and/or TEM2 bispecific antibodies

It is believed that agonistic anti-TREM2, or TREM2 bispecific antibodies may induce F-actin in microglial cells, macrophages, and dendritic cells.

Microglia, macrophages or dendritic cells and other cells of interest that are transduced with TREM2 or that express TREM2 are added to culture plates and then exposed to agonistic anti-TREM2, and/or TREM2 bispecific antibodies, or a control antibody. Cells are fixed, blocked, and then stained with Alexa Fluor 546-conjugated phalloidin (Molecular Probes) after 1 h and F-actin is labeled with a fluorescence dye. Images are collected by confocal laser scanning microscopy with a 40x objective lens (Leica). (JEM (2005), 201, 647–657).

Example 12: Induction of osteoclast production and increased rate of osteoclastogenesis by agonistic TREM2, DAP12, and/or TEM2/DAP12 bispecific antibodies

It is believed that agonistic anti-TREM2 and/or TREM2 bispecific antibodies may induce osteoclast production and increase the rate of osteoclastogenesis.
[0457] RAW264.7 cells that make osteoclasts or bone marrow-derived monocyte/macrophage (BMM) precursor cells are maintained in RPMI-1640 medium (Mediatech), or another appropriate medium, supplemented with 10% FBS (Atlantic Biologics, Atlanta, GA, USA) and penicillin-streptomycin-glutamine (Mediatech). TREM2B cDNA with a FLAG epitope added to the N terminus is inserted into the retroviral vector pMXpie upstream of an IRES, followed by an eGFP cDNA sequence. Cells are transfected with pMXpie-FLAG TREM2B, using Eugene 6 (Roche) according to manufacturer’s protocol. Cells are selected in puromycin (Sigma) at 2 μg/ml. Stable puromycin-resistant clones are screened for anti-FLAG M2 monoclonal antibody (Sigma) binding by using flow cytometry, and then subcloned and maintained on puromycin selection media.

[0458] RAW264.7 cells expressing TREM2B are seeded in 96-well plates with 3000 cells/well in alpha-MEM medium supplemented with 10% FBS, penicillin-streptomycin-glutamine, 50 ng/ml RANKL, and 20 ng/ml M-CSF. The medium is changed every 3 days, exposed to anti-TREM2 agonistic antibodies and the number of multinucleated (at least three nuclei) TRACP⁺ osteoclasts are counted and scored by light microscopy. To determine complexity and size, osteoclasts are counted by number of nuclei (>10 or 3–10 nuclei). The surface area of osteoclasts is also measured by using Image J software (NIH). In addition, expression levels of osteoclasts genes are determined. Total RNA is extracted from osteoclastogenic cultures at different time points using TRIzol reagent (Invitrogen). After first-strand cDNA synthesis using a SuperScript III kit (Invitrogen), real-time quantitative PCR reactions are performed for Nfatc1, Acp5, Ctsk, Calcr, and Cend1. Relative quantification of target mRNA expression is calculated and normalized to the expression of cyclophilin and expressed as (mRNA of the target gene/mRNA of cyclophilin) 3 X10⁶. (J. OF BONE AND MINERAL RESEARCH (2006), 21, 237–245; J Immunol 2012; 188:2612-2621).

[0459] Alternatively, BMM cells are seeded onto the plates in triplicate wells and treated with RANKL, M-CSF, and with an anti-TREM2, and/or TREM2 bispecific antibody, or an isotype-matched control monoclonal antibody. The medium is changed every 3 days until large multinucleated cells are visible. After 3 to 5 days in culture, cells are fixed with 3.7% formaldehyde in PBS for 10 min. Plates are then washed twice in PBS, incubated for 30 s in a
solution of 50% acetone and 50% ethanol, and washed with PBS. Cells are stained for tartrate-resistant acid phosphatase (TRAP) with a kit from Sigma (product 435). Multinucleated (more than two nuclei), TRAP-positive cells are then counted by light microscopy, as described (e.g., Peng et al., (2010) Sci Signal., 3(122): ra38).

Example 13: In vivo protection from EAE and cuprizone in a whole animal

[0460] Adult 7-9 week-old female C57BL/6 mice (obtained from Charles River Laboratories) are injected in the tail base bilaterally with 200 μl of an inoculum containing 100 μg of myelin oligodendrocyte glycoprotein peptide 35–55 (amino acids MEVGWYRSPFSRVHLYRNGK; Seqlab) and 1 mg of Mycobacterium tuberculosis H37 Ra (Difco) in incomplete Freund adjuvant (Difco). Pertussis toxin (200 ng; List Bio- logical Laboratories) is injected at day 0 and at day 2 after immunization. Clinical signs are scored as follows: 0, no clinical signs; 1, complete limp tail; 2, complete limp tail and abnormal gait; 3, one hind-limb paraparesis; 4, complete hindlimb paraparesis; and 5, fore- and hind-limb paralysis or moribund. Only mice having disease onset (clinical score of 1 or more) at day 14 are used for experiments. Agonistic anti-TREM2, and/or TREM2 bispecific antibodies are injected intraperitoneally or intravenously in EAE-diseased mice at the day of the first clinical symptoms or at any other desired time (PLoS Med (2007) 4(4): e124).

[0461] Young or aged wild-type (WT) mice are fed a standard diet (Harlan) containing 0.2 % cuprizone (CPZ) powdered oxalic bis(cyclohexylidenehydrazide) (Sigma-Aldrich) for 4, 6 or 12 weeks. For Histological and immunohistochemical analyses brains are removed after mouse perfusion with 4 % paraformaldehyde (PFA), fixed in 4 % PFA for 24 h, followed by immersion in 30 % sucrose for 24–48 h. To evaluate myelin integrity and damage, as well as cell proliferation and inflammation sections or mouse brain are stained with anti-MBP (1:100; Abcam, ab7349), -dMBP (1:2000; Millipore, ab5864), -β APP (1:100; Invitrogen, 51-2700), -SMI-31 (1:1000;Covance, smi-31R), -Iba1 (1:600; Wako, 019-19741), -BrdU (1:250; Abcam, ab1893), -GFAP (1:200; Invitrogen,13-0300), -iNOS (1:100; BD Pharmingen, 610329), -LPL(1:400, from Dr. G. Olivecrona) and -MHC II (1:100; BD Pharmingen, 553549). For behavioral effects of the antibodies, mice are analyzed for locomotor activity using transparent
polystyrene enclosures and computerized photobeam instrumentation. General activity variables (total ambulations, vertical rearings), along with indices of emotionality including time spent, distance traveled and entries, are analyzed. A battery of sensorimotor tests is performed to assess balance (ledge and platform), strength (inverted screen), coordination (pole and inclined screens) and initiation of movement (walking initiation). Motor coordination and balance are studied using a rotarod protocol (Cantoni et al., Acta Neuropathol (2015)129(3):429-47).

Example 14: Characterization of the therapeutic use of agonistic TREM2 and/or TREM2 bispecific antibodies in established animal models of traumatic brain injury

[0462] The therapeutic utility of agonistic anti-TREM2, and/or TREM2 bispecific antibodies is tested in established animal models of traumatic brain injury (Tanaka, Y et al. (2013) Neuroscience 231 49-60). For example, a model of traumatic brain injury that induces the activation of microglia and astrocytes is used. Eight or nine week-old male C57BL/6J WT mice or progranulin heterozygous mice are used (purchased from Charles River Laboratories or Jackson Laboratories). Mice are anesthetized by intraperitoneal administration of xylazine hydrochloride (8 mg/kg) and chloral hydrate (300 mg/kg) dissolved in sterile saline, and subsequently placed in a stereotaxic apparatus (Narishige, Tokyo, Japan). An incision is made in the scalp and the cranium is exposed. The periosteum is cleaned from the skull, a hole is drilled over the right cerebral hemisphere with a dental drill, and the duramater is removed with a needle tip. A stainless steel cannula, with a 0.5 mm outer diameter, is used to make a longitudinal stab wound in the right hemisphere. The cannula is positioned at 1.3 mm lateral to the midline, and 1 mm posterior to bregma, and introduced into the brain until the tip reaches a depth of 2 mm. The cannula is then shifted 2 mm caudally (bregma 3 mm), and then shifts back 2 mm rostrally to its initial position. Finally, the cannula is removed from the brain, and the scalp wound is sutured. Mice are then treated with agonistic anti-TREM2, and/or TREM2 bispecific antibodies according to standard procedures and then analyzed by histology and immunofluorescence staining and behavioral tests.
Example 15: Characterization of therapeutic use of agonistic TREM2 and/or TREM2 bispecific antibodies in a model of neuro-inflammation and neuron loss following toxin-induced injury

[0463] The therapeutic utility of agonistic anti-TREM2, and/or TREM2 bispecific antibodies is tested in a model of neuro-inflammation and neuron loss following toxin-induced injury (Martens, LH et al., (2012) The Journal of Clinical Investigation, 122, 3955). Three-month-old mice are treated with 4 intraperitoneal injections of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) per day for 2 days (4 μg/g body weight) (Sigma-Aldrich) or PBS. Mice are treated with agonistic anti-TREM2, and/or TREM2 bispecific antibodies according to standard protocols and then analyzed using Stereological counting to quantify dopamine neurons and microglia in the substantia nigra pars compacta (SNpc), as described.

Example 16: Characterization of the therapeutic use of agonistic TREM2 and/or TREM2 bispecific antibodies in animal models of aging, seizures, spinal cord injury, retinal dystrophy, frontotemporal dementia, and Alzheimer's disease

Example 17: Characterization of the therapeutic use of agonistic TREM2 and/or TREM2 bispecific antibodies in models of atherosclerosis

[0465] The therapeutic utility of agonistic anti-TREM2 and/or TREM2 bispecific antibodies is tested in models of atherosclerosis, as previously described (e.g., Lance, A et al., (2011) Diabetes, 60, 2285; and Kjolby, M et al., (2012) Cell Metabolism 12, 213-223).

Example 18: Characterization of the therapeutic use of agonistic TREM2 and/or TREM2 bispecific antibodies in a model of infection

[0466] The therapeutic utility of agonistic anti-TREM2 and/or TREM2 bispecific antibodies is tested in a model of infection. For example, Listeria monocytogenes or other infection in normal mice or progranulin heterozygous mice can be used, as previously described (e.g., Yin, F et al., (2009) J. Exp. Med, 207, 117-128).

Example 19: Characterization of the therapeutic use of agonistic TREM2 and/or TREM2 bispecific antibodies in a model of inflammatory diseases


Example 20: Screening for anti-TREM2 and/or TREM2 bispecific antibodies that induce phosphorylation of TREM2, DAP12, SYK, ERK, and AKT. which indicate activation of the PI3K pathway

[0468] Cells (J774, RAW 264.7, BMM cells, or osteoclasts) are removed from tissue culture dishes with PBS-EDTA, washed with PBS, and counted. J774 (40 × 10^6) or RAW 264.7 cells (10 × 10^6 BMM or osteoclasts) are incubated with an anti-TREM2 and/or TREM2 bispecific antibody or with an isotype-matched control antibody at 1 μg/10^6 cells for 20 min on ice or under other conditions. Cells are lysed in ice-cold radioimmunoprecipitation assay (RIPA) buffer for 20 min followed by centrifugation at 16,000 g for 10 min at 4°C to remove insoluble materials. The resulting supernatant is subjected to immunoprecipitation reactions with the indicated
antibodies (DAP12, ERK, or AKT) and protein A- or protein G-agarose (Sigma). The beads are extensively washed with RIPA buffer and the proteins are separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins are then transferred to nitrocellulose membranes by Western blotting, incubated with the appropriate antibodies (antibodies that specifically recognize the phosphorylated form of DAP12, ERK, or AKT) and visualized with the enhanced chemiluminescence (ECL) system (Pierce), as described (e.g., Peng et al., (2010) Sci Signal., 3(122): ra38).

Example 21: Screening for anti-TREM2, and/or TREM2 bispecific antibodies that induce calcium flux

BMM cells are washed twice with HEPES-containing buffer [20 mM HEPES (pH 7.3), 120 mM NaCl, 1 mM CaCl, 1 mM MgCl, 5 mM KCl, glucose (1 mg/ml), bovine serum albumin (1 mg/ml)] followed by incubation in 0.05% Pluronic F-127 (Invitrogen) and 1 μM Indo-1 AM (Invitrogen) for 20 min at 37°C. Cells are washed twice with HEPES buffer and are then stimulated with an anti-TREM2 and/or TREM2 bispecific antibody (16 μg/ml) or with a control antibody (16 μg/ml) and monitored by spectrophotometer (PTL Photon Technology International). The Indo-1 fluorescence emission is converted to calcium (Ca^{2+}) according to manufacturer’s instructions (e.g., Peng et al., (2010) Sci Signal., 3(122): ra38).

Example 22: Screening for anti-TREM2 and/or TREM2 bispecific antibodies that promote survival of osteoclasts and/or microglia

Murine Bone Marrow precursors are obtained by flushing tibial and femoral marrow cells with cold PBS. After one wash with PBS, erythrocytes are lysed using ACK Lysing Buffer (Lonza), washed twice with PBS and suspended at 0.5x10^6 cells/ml in complete RPMI media (10% FCS, Pen/Strep, Gln, neAA) with the indicated amounts of 50 ng/ml M-CSF to make macrophages or 10 ng/ml GM-CSF. For M2-type macrophages, 10 ng/ml IL-4 is added to the cultured cells. For M1-type macrophages, 50 ng/ml IFN-γ is added. In some experiment LPS or zymosan is added to the cell culture at day 5, at a concentration of 1 μg/ml-0.01 ng/ml. Recombinant cytokines were purchased by Peprotech. To analyze viability of BM derived macrophages, cells of the indicated genotype are prepared as above and cultured in graded
concentrations of MCSF. Cells are either plated at 10^7/200 μl in a 96-well plate (for viability analysis using a luciferase based-assay) or at 0.5x10^6/ml in a 6-well plate (for Trypan Blue exclusion cell count) in non-tissue culture treated plates. Media containing fresh M-CSF is added at day 3. At the indicated time points cells are gently detached from the plates with 3 mM EDTA and counted using a Burker chamber. In some experiments cells are also stained for FACS analysis using CD11b antibody and DAPI. Alternatively, cells are directly incubated with ToxGlo reagent (Promega) and luciferase activity is determined. In some experiments MCSF is withdrawn or not from the culture media at day 5 and cell viability is analyzed 36 hours later by FACS.

**[0471]** Mature osteoclast cell cultures are differentiated in 24-well dishes with RANKL and M-CSF. After 4 days, complete medium is substituted with serum-free medium to induce apoptosis. Cells are treated with RANKL, PBS, and an anti-TREM2 and/or TREM2 bispecific antibody, or an isotype-matched control antibody, during the overnight serum starvation. Cells are fixed in 1% paraformaldehyde and stained with a TUNEL-based kit (Millipore Corporation) according to manufacturer’s instructions. Apoptotic nuclei are counted with a Nikon TE2000-E microscope with 20x magnification. Results are expressed as the percentage of apoptotic cells relative to the total number of cells in six randomly selected fields of the two wells, as described (e.g., Peng et al., (2010) Sci Signal., 3(122): ra38). Similar assays are performed with primary microglial cells.

**Example 23: TREM2 increases the survival of macrophages and dendritic cells**

**[0472]** To evaluate the role of TREM2 in cell survival, wild-type (WT), TREM2 knock-out (KO), and TREM2 heterozygous (Het) macrophages and dendritic cells were cultured in the presence of inflammatory mediators, and cell survival was measured.

**[0473]** Murine bone marrow precursor cells from TREM2 WT, Het, and KO mice were obtained by flushing tibial and femoral marrow cells with cold PBS. After one wash with PBS, erythrocytes were lysed using ACK Lysing Buffer (Lonza), washed twice with PBS and suspended at 0.5x10^6 cells/ml in complete RPMI media (10% FCS, Pen/Strep, Gln, neAA) with the indicated amounts of 50ng/ml M-CSF to produce macrophages, or 10ng/ml GM-CSF to
produce dendritic cells. For M2-type macrophages, 10ng/ml IL-4 was added to the cultured cells. For M1-type macrophages, 50ng/ml IFN-γ was added. In some experiments LPS or zymosan was added to the cell culture at day 5 at a concentration range of 1μg/ml-0.01ng/ml. Recombinant cytokines were purchased from Peprotech.

[0474] To analyze viability of bone marrow-derived macrophages, cells were prepared as above and cultured in MCSF. Cells were either plated at 10⁵/200μl in a 96-well plate (for viability analysis using a luciferase based-assay) or at 0.5x10⁶/1ml in a 6-well plate (for Tripan Blue exclusion cell count) in non-tissue culture treated plates. Media containing fresh M-CSF was added at day 3. At the indicated time points cells were gently detached from the plates with 3mM EDTA and counted using a Burker chamber. For FACS analysis of live cells, macrophages were cultured either in 50ng/ml MCSF for 6 days (+MCSF) or in 50ng/ml MCSF for 4 days before MCSF was removed for an additional 36 hrs (-MCSF). Cells were stained using CD11b antibody and DAPI. For luciferase viability assays, cell viability was measured at day 5 of culture in graded concentrations of growth factors GMCSF (dendritic cells), MCSF (M1 macrophages), or MCSF+IL-4 (M2 macrophages). Cells were directly incubated with ToxGlo reagent (Promega) and luciferase activity (luminescence) was determined. For FACS analysis of viable macrophages cultured in the presence of inflammatory mediators IFN-γ, LPS, or zymosan, cells were collected at day 5 and stained using CD11b antibody and DAPI.

[0475] After 7 days of culture in MCSF, significantly higher numbers of viable (trypan blue excluded) TREM2 WT and Het (TREM2°/°) macrophages were observed than TREM2 KO (TREM2°/°) macrophages (FIG. 8A). FACS analysis revealed that WT macrophages treated with MCSF for either 6 (+MCSF) or 4 days (-MCSF) displayed increased survival compared to Het and KO macrophages, as indicated by a higher percentage of live (CD11b+DAPI-) cells (FIG. 8B). For luciferase assays, WT cells cultured in the presence of growth factors GMCSF (dendritic cells), MCSF (M1 macrophages), or MCSF+IL-4 (M2 macrophages) survived better than KO cells, as indicated by a higher luminescence reading across the range of growth factor concentrations (FIG. 8C). Based on these results, TREM2 agonistic antibodies may be able to increase the survival of macrophages and dendritic cells, while TREM2 antagonistic antibodies can decrease cell survival.
Example 24: Screening to determine which anti-TREM2, and/or TREM2 bispecific antibodies compete with ligand binding

It is believed that some anti-TREM2 agonistic antibodies may recognize the ligand-binding site on TREM2 and will compete with ligand binding on the TREM2 receptors. To determine which antibodies compete with binding of E.coli to Trem2. Inactivated E.coli with CellTracker DeepRed (ThermoFisher/ Invitrogen) were added to Trem2/Dap12 BWZ cells together with 10ug/ml of Trem2 antibodies and incubated for one hour on ice. Cells were then analyzed via FACS for binding of CellTracker labeled bacteria. TREM2 expression causes very strong bacteria binding to BWZ cells.

Example 25: TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 compete with TREM2 ligand for binding to human and mouse TREM2

[0476] The ability of agonist TREM2 antibodies to recognize the ligand-binding site on TREM2 was evaluated through competitive binding assays with E.coli cells expressing a putative TREM2 ligand.

[0477] E.coli were grown in 10ml LB media O/N, harvested by centrifuging, and washed twice in 10ml PBS. E. coli were then heat-inactivated by incubating in a 70°C water bath for 30min. E.coli were labeled with CellTracker DeepRed (ThermoFisher/ Invitrogen, 1uM final concentration) and subsequently washed thrice in 10ml PBS and resuspended in 1ml PBS at a concentration of 10^6/ml. For competition binding, bacteria were added to a mouse TREM2 and DAP12 expressing cell line (BWZ), or to a BW cell line expressing a human TREM2/DAP12 fusion protein, together with 10μg/ml of full-length agonist TREM2 antibodies and incubated for one hour on ice. Cells were analyzed via FACS for binding of CellTracker labeled bacteria to the cell lines.

[0478] TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 inhibited the binding of E.coli bacteria to both human and mouse cells, indicating competitive binding of the antibodies to the ligand-binding site on TREM2 (FIG. 9). Non-agonistic control TREM2 antibodies (Ab66
and Ab68) partially inhibited bacterial binding to human cells expressing TREM2, but did not inhibit binding to mouse TREM2, indicating that they are weaker competitors of ligand binding.

Example 26: Screening for anti-TREM2 and/or TREM2 bispecific antibodies that normalize TREM2/TYROBP-dependent changes in gene expression within the immune/microglia regulatory module

[0479] Microglial cells derived from mouse embryonic stem cells are genetically modified by lentiviral vectors to overexpress either full-length or a truncated version of Tyrobp that lacks both intracellular immunoreceptor tyrosine-based activation motif (ITAM) motifs. Microglia cells are also derived from mouse embryonic stem cells that are heterozygous for TREM2. To assess the genome-wide gene-expression changes in response to the perturbation of Tyrobp or TREM2, gene-expression data is derived from the RNA sequencing of mouse microglial macrophages or dendritic cells overexpressing: (1) vehicle, (2) full-length Tyrobp, or (3) dominant-negative truncated Tyrobp; or (4) overexpressing a knockdown construct for TREM2, such as SiRNA and cells which are heterozygous for TREM2 as well as from cells derived from TREM2 deficient mouse. Approximately 2,638 and 3,415 differentially expressed genes for the overexpression of full-length Tyrobp and truncated Tyrobp are identified, respectively (Zhang et al., (2013) Cell 153, 707–720). Approximately 99% of the differentially expressed genes from the microglia overexpressing intact Tyrobp are downregulated compared to the control vehicle. For example, 658 genes, related to the vacuole/autophagy, as well as genes involved with RNA metabolism and cell-cycle mitosis are downregulated by active Tyrobp, but upregulated in cells expressing dominant-negative truncated Tyrobp. Conversely, some 2,856 genes for the vacuole/autophagy pathway and for mitochondrion are selectively upregulated in microglia expressing the dominant-negative truncated Tyrobp.

[0480] Agonistic anti-TREM2, and/or TREM2 bispecific antibodies are screened for their ability to elicit gene expression profiles similar to that observed in normal microglial cells and in microglial cells overexpressing intact Tyrobp in cells that express dominant-negative truncated Tyrobp (Zhang et al., (2013) Cell 153, 707–720), in cells that express the knockdown construct
for TREM2, or in cells that are heterozygous for TREM2. Antibodies that are capable of changing the gene expression network are selected.

Example 27: TREM2 antibodies Ab1, Ab9, Ab22, Ab45, and Ab65 induce DAP12 phosphorylation in mouse macrophages

[0481] TREM2 signals through DAP12, leading downstream to activation of PI3K and other intracellular signals. The ability of agonist TREM2 antibodies to induce DAP12 activation was determined by culturing mouse macrophages and measuring the phosphorylation state of DAP12 protein in cell extracts.

[0482] Before stimulation with mAbs, mouse wild-type (WT) bone marrow-derived macrophages (BMDM) and TREM2 knockout (KO) BMDM were starved for 4h in 1% serum RPMI. 15 x 10^6 cells were incubated in ice for 15 min with full-length agonistic or control antibodies. Cells were washed and incubated at 37°C for the indicated period of time in the presence of goat anti-human IgG. After stimulation, cells were lysed with lysis buffer (1% v/v NP-40%, 50 Mm Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1.5 mM MgCl2, 10% glycerol, plus protease and phosphatase inhibitors), followed by centrifugation at 16,000 g for 10 min at 4°C to remove insoluble materials. Cell lysate was immunoprecipitated with a second TREM2 antibody (R&D Systems). Precipitated proteins were fractionated by SDS-PAGE, transferred to PVDF membranes, and probed with anti-phosphotyrosine Ab (4G10, Millipore). The membrane was stripped and reprobed with anti-DAP12 antibody (Cells Signaling, D7G1X). Each cell lysate used for TREM2 immunoprecipitations contained an equal amount of proteins, as indicated by a control Ab (anti-actin, Santa Cruz).

[0483] DAP12 co-precipitated with TREM2 and was phosphorylated in WT macrophages incubated with agonist TREM2 antibodies Ab1, Ab9, Ab22, Ab45, and Ab65 (FIG. 10A and 10B). Conversely, no DAP12 phosphorylation was observed in TREM2 KO (TREM2^-^-) macrophages incubated with antibodies Ab1, Ab9, Ab22, or Ab45 (FIG. 10B). These results demonstrate that agonistic antibodies Ab1, Ab9, Ab22, and Ab45 induce phosphorylation of TREM-2-associated DAP12 in a TREM-2 specific manner, as DAP12 phosphorylation is absent in TREM-2 deficient BMDM.
Example 28: TREM2 decreases the expression of inflammatory cell surface markers on mouse macrophages

In order to determine the role of TREM2 in inflammatory marker expression, mouse wild-type (WT), TREM2 knock-out (KO), and TREM2 heterozygous (Het) macrophages were cultured with various inflammatory mediators, and the expression of surface markers CD86 and CD206 were measured.

Macrophages isolated from WT, KO, and Het mice were plated and allowed to adhere for 4 h at 37°C, and TLR agonists LPS (Salmonella abortus equi) and zymosan (Saccharomyces cerevisiae) were added at concentrations ranging from 0.01-100ng/ml (LPS) or 0.01-10μg/ml (zymosan). Alternatively, macrophages isolated from WT, KO, and Het mice were cultured in the presence of the cytokines IL-4 (10ng/ml) or IFN-γ (0.5-50ng/ml). FACS analysis of CD86 and CD206 was performed on a BD FACS Canto 48 hours later. Data analysis was performed with FlowJo (TreeStar) software version 10.0.7.

Wild-type (WT) macrophages treated with the inflammatory mediators IFN-γ (FIG. 11A), LPS, or Zymosan (FIG. 11B) expressed lower levels of the inflammatory receptor CD86 but not of the receptor CD206 compared TREM2 KO (TREM2−/−) macrophages. Similarly, Het (TREM2−/+) macrophages treated with IFN-γ expressed lower levels of CD86 but not of CD206 compared to TREM2 KO macrophages (FIG. 11A). Based on these results, TREM2 agonistic antibodies may reduce expression of inflammatory receptors on macrophages.

Example 29: TREM2 modulates phagocytosis

TREM2 signaling is involved in phagocytosis pathways, including bacterial clearance from the lung and phagocytosis of apoptotic neurons. The role of TREM2 in phagocytosis was evaluated by measuring the ability of wild-type (WT) and TREM2 knock-out (KO) mouse macrophages to phagocytose E. coli cells and apoptotic cells.

WT and TREM2 KO BMDM were starved in 1% serum RPMI or kept in culture in the presence of MCSF (50ng/ml) overnight. The following day cells were plated at 2x10^5 in 96 well plates (round bottom non tissue culture), in the presence or absence of MCSF. Target cells
(CCL119) were cultured overnight with 0.5 μM staurosporine to induce apoptosis. The day after cells were washed and labeled with 20ng/ml of pHrodo-SE (Invitrogen). Apoptotic cells or bioparticle E. coli (pHrodo, Invitrogen) were added and the cells were incubated at 37°C for 1h and 30 min. The assay was stopped on ice. After washing with cold PBS, cells were stained for CD11b (pacific blue- CD11b, BD) and analyzed by FACS. In all samples, BMDM were distinguished from apoptotic cells and beads by CD11b staining, and gates were drawn based on effector cells cultured without target cells. For negative controls, effector cells were incubated during the entire assay with cytochalasin D (2μM, SIGMA). Phagocytosis was quantified as (percent or MFI of PhRodo positive cells) - (percent or MFI of PhRodo positive cytochalasin D treated negative control cells).

[0489] Wild-type (WT) macrophages cultured with MCSF displayed less phagocytosis of apoptotic cells and E. coli cells as compared to TREM2 KO (TREM2<sup>−/−</sup>) macrophages, as indicated by a lower percentage or lower MFI of pHrodo+ cells (FIG. 12). Conversely, WT macrophages cultured without MCSF displayed increased phagocytosis of apoptotic cells and E. coli cells as compared to TREM2 KO macrophages, as indicated by a higher percentage of pHrodo+ cells (FIG. 12). Based on these results, TREM2 agonistic antibodies may enhance phagocytosis in the absence of MCSF and reduce phagocytosis in the presence of MCSF.

Conversely, it is believed that TREM2 antagonistic antibodies can enhance phagocytosis in the presence of MCSF and reduce phagocytosis in the absence of MCSF.

**Example 30: Epitope mapping of TREM2 antibodies and comparison of TREM2 antibodies to reference TREM2 antibodies**

[0490] TREM2 antibodies were tested for their ability to bind 15 or 25 mer peptides spanning the entire human and mouse TREM2. The TREM2 antibodies were also compared to a reference TREM2 antibody by determining their TREM2 binding region.

[0491] Linear 15-mer peptides were synthesized based on the sequence of human or mouse TREM2, with a 14 residue overlap. In addition, linear 25-mer peptides were synthesized based on sequence of human or mouse TREM2 with a 1 residue shift. The binding of TREM2 antibodies to each of the synthesized peptides was tested in an ELISA based method. In this
assay, the peptide arrays were incubated with primary antibody solution (overnight at 4°C). After washing, the peptide arrays were incubated with a 1/1000 dilution of an antibody peroxidase conjugate (SBA, cat. nr. 2010-05) for one hour at 25°C. After washing, the peroxidase substrate 2,2'- azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 2 μl/ml of 3% H2O2 were added. After one hour, the color development was measured. The color development was quantified with a charge coupled device (CCD) camera and an image processing system.

[0492] To evaluate antibody binding regions, human TREM2-Fc was incubated with immobilized full-length agonist TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, Ab63, Ab65, or Ab87, and TREM2 antibody MAB17291 (R&D Systems) was subsequently added. Epitope binning of the antibodies was performed on a Forte Bio Octet Red384 system (Pall Forte Bio Corporation, Menlo Park, CA) using a standard sandwich format binning assay (see Estep et al., (2013) MAb5 5(2):270-8). Control anti-target IgG was loaded onto AHQ sensors and unoccupied Fe-binding sites on the sensor were blocked with a non-relevant human IgG1 antibody. The sensors were then exposed to 100 nM target antigen followed by a second anti-target antibody. Data was processed using ForteBio’s Data Analysis Software 7.0. Additional binding by the second antibody after antigen association indicates an unoccupied epitope (non-competitor), while no binding indicates epitope blocking (competitor).

[0493] Antibodies Ab1, Ab9, Ab28, and Ab29 yielded strong and robust binding exclusively for peptides from Set1 and Set2, which are derived from the sequence of human TREM2. All four antibodies bound peptides that contain the region between amino acid residues 139-146 of human TREM2 (139GLWFPGE146) (FIG. 13A). The epitope region recognized by Ab1, Ab9, Ab28, and Ab29 corresponds to amino acid residues 139-146 of SEQ ID NO: 1 and has the amino acid sequence of: GLWFPGE (SEQ ID NO: 237).

[0494] Antibodies Ab45 and Ab65 bound only to 25-mer peptides from Set2 and Set4. Both antibodies recognized a highly conserved region of TREM2 near its C-terminus between amino acid residues 139-153 of the human TREM2 (139DLWFPGESESFEDEA153) and mouse TREM2 (139DLWVPEESSSFEGA153) (FIG. 13B). The epitope region recognized by Ab45 and Ab65
corresponds to amino acid residues 139-153 of SEQ ID NO: 1 and has the amino acid sequence of: DLWFPGESESFEDA (SEQ ID NO: 238).

[0495] The epitope region recognized by reference antibody MAB17291 was also determined. Reference antibody MAB17291 was found to recognize a first peptide that contains the region between amino acid residues 130-144 of human TREM2 (130ADPLDHRDAGPLWFP144), and a second peptide that contains the region between amino acid residues 158-170 of human TREM2 (158 SISRSLLEGEPFP170). The epitope regions recognized by MAB17291 correspond to amino acid residues 130-144 and 158-170 of SEQ ID NO: 1 and have the amino acid sequences of: ADPLDHRDAGPLWFP (SEQ ID NO: 240) and SISRSLLEGEPFP (SEQ ID NO: 241).

[0496] Reference antibody MAB17291 was able to simultaneously bind TREM2 with antibody Ab1, Ab9, Ab14, Ab22, Ab45, or Ab65, but not with antibody Ab63 or Ab87 (FIG. 14). These results demonstrate that agonist TREM2 antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 bind different regions of the TREM2 protein than does reference antibody MAB17291.

Example 31: Summary of TREM2 antibody functional studies.

[0497] Table 6 summarizes results of the functional studies described in Examples 3, 9, 25, and 27 above. Antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 demonstrated induction of CD83 and CD86 on human dendritic cells (hDC), with higher induction observed with plate-bound antibody compared to cross-linked soluble antibody (values in table represent percentage of CD83+CD86+ cells). Antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 induced variable levels of Syk phosphorylation in human dendritic cells (hDC), mouse dendritic cells (mDC), and mouse macrophages (mMac), and were able to mimic ligand binding (blocking bacterial binding) to human and mouse TREM2.

**Table 6: TREM2 Antibody Functional Studies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Induction of CD86 &amp; CD83 Human hDC plate</th>
<th>Induction of CD86 &amp; CD83 hDC solution</th>
<th>Induction Phospho Syk hDC</th>
<th>Induction Phospho Syk mDC</th>
<th>Induce Phospho TREM2 and DAPI/2 mM Ac</th>
<th>Blocking Bacterial binding hTREM2</th>
<th>Blocking Bacterial binding mTREM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab1</td>
<td>78.7</td>
<td>3.0</td>
<td>+/-</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Ab9</td>
<td>79.3</td>
<td>5.6</td>
<td>-/+</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Ab14</td>
<td>78.7</td>
<td>1.9</td>
<td>+</td>
<td>++</td>
<td>N/D</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Ab22</td>
<td>74.5</td>
<td>1.2</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Ab45</td>
<td>65.5</td>
<td>4.3</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Ab65</td>
<td>74</td>
<td>1.7</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
</tbody>
</table>

sf-3499172 -209-
Example 32: Analysis of tumor growth in TREM2 deficient mice

[0498] Groups of 10 TREM2 wild-type (WT), TREM2 heterozygous (HET), and TREM2 knock-out (KO) mice (sex and age-matched littermates, 8 weeks old (+/- 2 weeks)) are challenged subcutaneously with tumor cells (for example $1 \times 10^5$ - $1 \times 10^6$ MC38 colon carcinoma, Lewis Lung carcinoma, or B16 melanoma cells) suspended in 100ul PBS. Animals are anesthetized with isoflurane prior to implant. Tumor growth is monitored with a caliper biweekly to measure tumor growth starting at day 4. The endpoint of the experiment is a tumor volume of 2000 mm$^3$ or 60 days. Tumor growth and % survival are the outcome measures. Reduced tumor take and growth rate and a reduced number of tumor infiltrating immune suppressor macrophages indicate increased effector T cell influx into the tumor in TREM2 KO mice.

[0499] To determine the number of infiltrating tumor associated immune suppressor macrophages and T cells, groups of 6-8 sex and age-matched littermates are used. 8 week old (+/- 2 weeks) WT-HET-KO littermates are challenged subcutaneously with tumor cells (e.g. $1 \times 10^5$ - $1 \times 10^6$ MC38, Lewis Lung, or B16 cells) suspended in 200ul Matrigel (Matrigel Matrix Growth Factor Reduced; BD). Animals are anesthetized with isoflurane prior to implant. 7 and 10 days after tumor injection, the matrigel plug is resected, incubated for 1 hour at 37°C with 1 mg/ml Collagenase D (Sigma), dissociated to obtain a single-cell suspension, and filtered through a cell strainer. To determine the amount of T cells recruited in the tumor and the ratio between effector T cells and regulatory T cells, 5x10$^6$ cells are stained with anti-CD45.2 PercpCy5.5, anti-CD3-FITC, anti-CD8-PECY7, anti-CD4-APC, anti-FoxP3-PE (BD), and DAPI. To determine the amount of monocyte/macrophage lineage cells recruited into the tumor, 5x10$^6$ cells are stained with anti-CD45.2 PercpCy5.5, anti-CD11b-PECY7, anti-F4/80-FITC, anti-Ly6C/G-APC, anti-CD86-PE, and DAPI. Cells are acquired on a BD FACS Canto. Data analysis is performed with FlowJo (TreeStar) software version 10.0.7.
Example 33: Analysis of the anti-cancer effect of TREM2 antagonistic antibodies

Groups of 10 C57Bl6/NTac mice at 8 weeks (+/- 2 weeks) of age are challenged subcutaneously with tumor cells (e.g. 1x10^5 to 1x10^6 MC38, Lewis Lung, or B16 cells) suspended in 100μl PBS. Animals are anesthetized with isoflurane prior to implant. Starting at day 2, groups of mice are injected i.p. every 3 days for 4 doses with 200μg of each of antagonistic anti-TREM2 antibodies, such as those described in Examples 38 and 40. Tumor growth is monitored with a caliper biweekly to measure tumor growth starting at day 4. The endpoint of the experiment is a tumor volume of 2000 mm^3 or 60 days. Tumor growth and % survival are the outcome measures. Reduced tumor take and growth rate, reduced number of tumor infiltrating immune suppressor macrophages, and increased effector T cell influx into the tumor indicate the anti-cancer effects of blocking anti-TREM2 antibodies.

Example 34: Analysis of additive anti-tumor effect of combination therapy that combines TREM2 antibodies with antibodies against inhibitory checkpoint proteins or inhibitory cytokines/chemokines and their receptors

Groups of 15 C57Bl6/NTac mice at 8 weeks (+/- 2 weeks) of age are challenged subcutaneously with tumor cells as described in Example 35. Animals are anesthetized with isoflurane prior to implant. Starting at day 2, mice are injected i.p. every 3 days for 4 doses with 200μg anti-TREM2 antibodies alone or in combination with antibodies against checkpoint proteins (e.g. anti-PDL1 mAb clone 10F.9G2 and/or anti-CTLA4 mAb clone UC10-4F10-11) at day 3, 6, and 9. Treatment groups include anti-TREM2; anti-CTLA4; anti-PDL1; anti-TREM2+anti-CTLA4; anti-TREM2+anti-PDL1; and isotype control. Tumor growth is monitored with a caliper biweekly to measure tumor growth starting at day 4. The endpoint of the experiment is a tumor volume of 2000 mm^3 or 60 days. Tumor growth and % survival are the outcome measures. A decrease in tumor growth and an increase in % survival with combination therapy indicate that anti-TREM2 antibodies have additive or synergistic therapeutic effects with anti-checkpoint antibodies. Antagonistic antibodies against checkpoint molecules include antibodies against PDL1, PDL2, PD1, CTLA4, B7-H3, B7-H4, HVEM, BTLA, KIR, GAL9,
TIM3, A2AR, LAG-3, and Phosphatidyl Serine. Antagonist antibodies against inhibitory cytokines include antibodies against CCL2, CSF-1, and IL-2.

Example 35: Analysis of additive anti-tumor effect of combination therapy that combines TREM2 antibodies with antibodies that activate stimulatory checkpoint proteins

[0502] Groups of 15 C57Bl6/NTac mice at 8 weeks (+/- 2 weeks) of age are challenged subcutaneously with tumor cells as described in Example 35. Animals are anesthetized with isoflurane prior to implant. Starting at day 2, mice are injected i.p. every 3 days for 4 doses with 200ug anti-TREM2 antibodies alone or in combination with agonistic antibodies that activate stimulatory checkpoint proteins (e.g. OX40 or ICOS mAb) at day 3, 6, and 9. Tumor growth is monitored with a caliper biweekly to measure tumor growth starting at day 4. The endpoint of the experiment is a tumor volume of 2000 mm³ or 60 days. Tumor growth and % survival are the outcome measures. A decrease in tumor growth and an increase in % survival with combination therapy indicate that anti-TREM2 antibodies have additive or synergistic therapeutic effects with stimulatory checkpoint antibodies. Stimulatory checkpoint antibodies include agonistic/stimulatory antibodies against CD28, ICOS, CD137, CD27, CD40, and GITR.

Example 36: Analysis of additive anti-tumor effect of combination therapy that combines TREM2 antibodies with stimulatory cytokines

[0503] Groups of 15 C57Bl6/NTac mice at 8 weeks (+/- 2 weeks) of age are challenged subcutaneously with tumor cells as described in Example 35. Animals are anesthetized with isoflurane prior to implant. Starting at day 2, mice are injected i.p. every 3 days for 4 doses with 200ug anti-TREM2 antibodies alone or in combination with stimulatory cytokines (e.g. IL-12, IFN-a). Tumor growth is monitored with a caliper biweekly to measure tumor growth starting at day 4. The endpoint of the experiment is a tumor volume of 2000 mm³ or 60 days. Tumor growth and % survival are the outcome measures. A decrease in tumor growth and an increase in % survival with combination therapy indicate that anti-TREM2 antibodies have additive or synergistic therapeutic effects with immune-stimulatory cytokines. Stimulatory cytokines include IFN-a/b, IL-2, IL-12, IL-18, GM-CSF, and G-CSF.
Example 37: Analysis of ability of TREM2 antibody Fabs to stimulate viability of innate immune cells

[0504] The agonistic functionality of plate bound, cross-linked anti-TREM2 antibody Fab fragments derived from antibodies Ab22, Ab45, and Ab65 was evaluated in innate immune cells (e.g., macrophages).

[0505] Wild-type (WT) and TREM2 knock-out (KO) mouse bone marrow derived macrophages were cultured in the presence of M-CSF and plate bound TREM2 antibody Fabs, and cell viability was measured.

[0506] Macrophages isolated from the bone marrow of WT and KO mice were plated on non-tissue-culture-treated 96-well plates, pre-coated with either 12.5nM or 100nM of cross-linked Ab22, Ab45, or Ab65 Fabs. Cells were cultured for 48 hours in the presence of 10ng/ml M-CSF. Analysis of viability was performed using Cell Titer Glo kit (Promega). Plates were read with a BioTek Synergy Microplate Reader using GEN5 2.04 software.

[0507] Cross-linked TREM2 Fab fragments derived from antibodies Ab22, Ab45, and Ab65 increased the number of viable mouse bone marrow-derived macrophages compared to isotype control Fab Ab88, as indicated by a higher % increased survival (FIG. 15). This enhancement in cell viability was not observed in KO mouse macrophages, with the exception of Ab65. These data indicate that the biological activity of Ab22 and Ab45 is TREM2 specific, and that plate bound, cross-linked Ab22 and Ab45 Fab fragments function as agonists to increase the survival of macrophages cultured in M-CSF.

Example 38: Analysis of ability of TREM2 antibodies to decrease survival of innate immune cells

[0508] The antagonistic functionality of both soluble, non-cross-linked anti-TREM2 antibody Fab fragments derived from antibodies and soluble, full-length anti-TREM2 antibodies was evaluated in innate immune cells (e.g., macrophages).
Wild-type (WT) and TREM2 knock-out (KO) mouse bone marrow derived macrophages were cultured in the presence of M-CSF and soluble TREM2 antibody Fabs or soluble full-length antibodies, and cell viability was measured.

Macrophages isolated from the bone marrow of WT and KO mice were plated on non-tissue-culture-treated 96-well plates in the presence of 20ng/ml M-CSF and increasing amounts of the indicated soluble, non-cross-linked TREM2 antibody Fabs derived from antibodies Ab22, Ab45, and Ab65, or soluble, full-length antibodies Ab9, Ab14, Ab22, Ab45, and Ab65. Each condition was plated in triplicate. Analysis of viability was performed using Cell Titer Glo kit (Promega) 3 days later. Plates were read with a BioTek Synergy Microplate Reader using GEN5 2.04 software.

In FIG. 16, the “NT” dotted line indicates the average cell viability obtained with untreated macrophages (no antibody added). The “no MCSF” dotted line indicates the average cell viability obtained when macrophages were cultured in the absence of M-CSF.

When macrophage cell viability was evaluated with soluble, non-cross-linked TREM2 antibody Fabs, the results indicated that soluble, non-cross-linked TREM2 Fab fragments derived from antibody Ab45 decreased cell viability (FIG. 16A). In contrast, soluble, non-cross-linked TREM2 Fab fragments derived from antibody Ab22 did not inhibit viability and had an effect comparable to the isotype control Ab99 (FIG. 16A). Soluble, non-cross-linked TREM2 Fab fragments derived from antibody Ab65 partially inhibited cell viability (FIG. 16A). The results demonstrate that the soluble, non-cross-linked Fab derived from TREM2 antibodies Ab45 and Ab65 can function as antagonists and inhibit the survival of macrophages in vitro.

When macrophage cell viability was evaluated with soluble, full-length antibodies, antibodies Ab14, Ab45, Ab65, and Ab9 decreased cell viability (FIG. 16B). Soluble, full-length antibody Ab22 had an effect on cell viability that was nearly comparable to that of isotype control Ab91 (FIG. 16B). The results demonstrate that soluble, full-length TREM2 antibodies Ab14, Ab45, Ab65, and Ab9 can function as antagonists, when not cross-linked or clustered, and inhibit the survival of macrophages.
The results of these experiments indicate that TREM2 antibodies Ab45, Ab65, and Ab9, in the absence of clustering, can inhibit the survival of innate immune cells such as macrophages. In contrast, TREM2 antibody Ab22, even in the absence of clustering, does not inhibit the survival of innate immune cells such as macrophages.

Example 39: Analysis of the ability of TREM2 antibodies to induce TREM2-dependent genes

The ability of plate bound full-length anti-TREM2 antibodies Ab1, Ab9, Ab10, Ab14, Ab15, Ab17, Ab18, Ab20, Ab22, Ab24, Ab25, Ab28, Ab29, Ab45, Ab54, Ab51, Ab64, Ab65, and Ab66 to activate TREM2-dependent genes was evaluate using a luciferase reporter gene under the control of an NFAT (nuclear factor of activated T-cells) promoter.

A cell line derived from mouse thymus lymphoma T lymphocytes BW5147 G.1.4 (ATCC® TIB48) was infected with mouse Trem2 and Dap12, and with Cignal Lenti NFAT-Luciferase virus (Qiagen). Full-length anti-TREM2 antibodies were plate bound at 10ug/ml in DPBS on tissue-culture treated clear bottom white 96well plates (100ul/well), overnight at 4°C. Wells were rinsed thrice with DPBS and subsequently were plated at 100,000 cells/well in media with 1% serum. As a positive control for signaling, PMA (0.05ug/ml) and ionomycin (0.25uM) were added together. Cells were incubated for 6 hours and luciferase activity was measured by adding OneGlo Reagent (Promega) to each well and incubating 3min at RT on a plate shaker. Luciferase signal was measured using a BioTek plate reader.

As shown in FIG. 17A and FIG. 17B, anti-TREM2 antibodies Ab1, Ab9, Ab10, Ab14, Ab15, Ab17, Ab20, Ab22, Ab45, Ab54, Ab64, Ab65, and Ab66 increased luciferase activity, indicating that the antibodies were able to induce TREM2-dependent gene transcription. As shown in FIG. 17C, plate bound phosphatidylinserine (PS) induces TREM2-dependent gene expression. It is believed that PS is a natural ligand of TREM2. Thus, the results in FIG. 17 indicate that agonist anti-TREM2 antibodies can mimic a natural ligand of TREM2.

Example 40: Analysis of the ability of TREM2 antibodies to inhibit TREM2-dependent genes

The ability of soluble, full-length anti-TREM2 antibodies Ab9, Ab14, Ab22, Ab45, and Ab65 to inhibit TREM2-dependent genes was evaluate using a luciferase reporter gene under the control of an NFAT (nuclear factor of activated T-cells) promoter.
[0519] A cell line derived from mouse thymus lymphoma T lymphocytes BW5147.G.1.4 (ATCC® TIB48™) was infected with mouse Trem2 and Dap12, and with Cignal Lenti NFAT-Luciferase virus (Qiagen). Soluble, full-length anti-TREM2 antibodies were added at increasing concentration to the cells. Cells were incubated for 6 hours at 37°C and luciferase activity was measured using OneGlo Reagent (Promega).

[0520] The cells display tonic TREM2-dependent signaling due to either the presence of an endogenous ligand or to spontaneous receptor aggregation, which leads to TREM2 signaling.

[0521] The dotted line in FIG. 18 indicates the levels of TREM2 activity without stimulation.

[0522] As shown in FIG. 18, soluble, full-length anti-TREM2 antibodies Ab9, Ab14, Ab45, and Ab65 were able to inhibit tonic, TREM2-dependent gene expression. In contrast, soluble, full-length anti-TREM2 antibody Ab22 did not appear to block tonic TREM2 signaling (FIG. 18).

Example 41: Summary of TREM2 antibody agonistic and antagonistic activity

[0523] Table 7 summarizes results of the functional studies described in Examples 37-39 above. Antibodies Ab1, Ab9, Ab14, Ab22, Ab45, and Ab65 demonstrated agonistic activity in activating TREM2-dependent gene expression using either a luciferase reporter gene (FIG. 17A) or a beta-GAL reporter gene (Table 7). As indicated in Table 7, Ab1 and Ab65 showed an increased level of gene induction, compared to Ab9, Ab22, and Ab45 when the beta-GAL reporter gene was used (Table 7). Antibodies Ab22, Ab45, and Ab65 also demonstrated agonistic activity in stimulating cell survival of innate immune cells (FIG. 15). Table 7 further summarizes results demonstrating the antagonistic effects of soluble, non-cross-linked antibodies Ab9, Ab14, Ab45, and Ab65 in inhibiting cell survival of innate immune cells (FIG. 16). In contrast, soluble, non-cross-linked antibody Ab22 had minimal antagonistic activity in inhibiting cell survival (FIG. 16).
Table 7: TREM2 Antibody Functional Studies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Luciferase Agonistic antibody activity</th>
<th>betaGAL Agonistic antibody activity</th>
<th>Survival Agonistic antibody activity</th>
<th>Luciferase Antagonistic antibody activity in solution/agonistic format</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab1</td>
<td>++++</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab9</td>
<td>++++</td>
<td>+</td>
<td>ND</td>
<td>+++</td>
</tr>
<tr>
<td>Ab14</td>
<td>++++</td>
<td>ND</td>
<td>ND</td>
<td>+++</td>
</tr>
<tr>
<td>Ab22</td>
<td>++++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Ab45</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Ab65</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Isotype control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Example 41: Summary of binding and functional studies for further TREM2 antibodies

Table 8 summarizes the results of binding and functional studies for further full-length TREM2 antibodies generated as described in Example 1. The results below were obtained using the methods described in Examples 1 and 9.

Table 8: Binding and functional studies for other TREM2 Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IgG Kd hTREM2-Fc (M) Avid</th>
<th>IgG Kd hTREM2-His (M) 1</th>
<th>IgG Kd mTREM2-Fc (M) Avid</th>
<th>mTREM2 Cell binding MFI</th>
<th>hTREM2 Cell binding MFI</th>
<th>Phospho Syk hDC</th>
<th>Phospho Syk mDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab2</td>
<td>5.92E-10</td>
<td>2.41E-08</td>
<td>N.B.</td>
<td>1432</td>
<td>1407</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab3</td>
<td>4.90E-10</td>
<td>1.42E-08</td>
<td>N.B.</td>
<td>488</td>
<td>1693</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab4</td>
<td>9.71E-10</td>
<td>P.F.</td>
<td>N.B.</td>
<td>295</td>
<td>1343</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab5</td>
<td>6.97E-10</td>
<td>3.00E-08</td>
<td>N.B.</td>
<td>310</td>
<td>1336</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Ab6</td>
<td>4.44E-09</td>
<td>P.F.</td>
<td>8.51E-09</td>
<td>228</td>
<td>816</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab7</td>
<td>1.05E-09</td>
<td>3.34E-08</td>
<td>1.11E-09</td>
<td>10934</td>
<td>1249</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab8</td>
<td>7.23E-10</td>
<td>1.50E-08</td>
<td>N.B.</td>
<td>523</td>
<td>1623</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab10</td>
<td>5.44E-10</td>
<td>4.79E-09</td>
<td>P.F.</td>
<td>11486</td>
<td>1788</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ab11</td>
<td>9.34E-10</td>
<td>7.20E-09</td>
<td>1.19E-09</td>
<td>5495</td>
<td>1909</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab12</td>
<td>1.19E-09</td>
<td>2.68E-09</td>
<td>7.91E-09</td>
<td>1552</td>
<td>1930</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Ab13</td>
<td>1.49E-09</td>
<td>6.67E-08</td>
<td>1.71E-09</td>
<td>3246</td>
<td>971</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab15</td>
<td>6.05I-10</td>
<td>3.03I-09</td>
<td>2.80I-09</td>
<td>4755</td>
<td>1611</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Ab16</td>
<td>1.09I-09</td>
<td>2.47I-09</td>
<td>1.36I-09</td>
<td>6382</td>
<td>1545</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Ab17</td>
<td>7.92I-10</td>
<td>3.46I-09</td>
<td>1.67I-09</td>
<td>10312</td>
<td>1604</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Ab18</td>
<td>5.13I-10</td>
<td>6.77I-09</td>
<td>N.B.</td>
<td>5164</td>
<td>1924</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Ab19</td>
<td>4.65I-10</td>
<td>4.49I-09</td>
<td>N.B.</td>
<td>2304</td>
<td>1993</td>
<td>++</td>
<td>ND</td>
</tr>
<tr>
<td>Ab20</td>
<td>6.58I-10</td>
<td>3.62I-09</td>
<td>N.B.</td>
<td>11245</td>
<td>1556</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Ab22</td>
<td>9.67I-10</td>
<td>P.F.</td>
<td>6.83I-09</td>
<td>919</td>
<td>2653</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab24</td>
<td>4.79I-10</td>
<td>P.F.</td>
<td>9.32I-09</td>
<td>2760</td>
<td>1882</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Ab25</td>
<td>6.84I-10</td>
<td>4.51I-09</td>
<td>5.98I-09</td>
<td>2497</td>
<td>1884</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Ab26</td>
<td>4.81I-10</td>
<td>3.60I-09</td>
<td>N.B.</td>
<td>2551</td>
<td>1421</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Antibody</td>
<td>IgG K&lt;sub&gt;e&lt;/sub&gt; hTREM2-Fe (M) Avid</td>
<td>IgG K&lt;sub&gt;d&lt;/sub&gt; hTREM2- HIS (M) t</td>
<td>IgG K&lt;sub&gt;e&lt;/sub&gt; mTREM2-Fc (M) Avid</td>
<td>mTREM2 Cell binding MFI</td>
<td>hTREM2 Cell binding MFI</td>
<td>Phospho Syk hDC</td>
<td>Phospho Syk mDC</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------</td>
<td>----------------------------------</td>
<td>----------------------------------</td>
<td>------------------------</td>
<td>------------------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Ab27</td>
<td>1.33E-09 P.F.</td>
<td>6.39E-09</td>
<td>2163</td>
<td>1316</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab28</td>
<td>9.15E-10 P.F.</td>
<td>7.45E-09</td>
<td>5248</td>
<td>1487</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Ab29</td>
<td>2.36E-09 P.F.</td>
<td>3.27E-09</td>
<td>4518</td>
<td>1193</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Ab30</td>
<td>1.32E-09 P.F.</td>
<td>N.B.</td>
<td>399</td>
<td>1421</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab31</td>
<td>4.51E-10 6.82E-09</td>
<td>N.B.</td>
<td>551</td>
<td>1760</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab32</td>
<td>9.95E-10 2.48E-08</td>
<td>1.35E-08</td>
<td>756</td>
<td>1202</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab33</td>
<td>1.42E-09 1.60E-08</td>
<td>N.B.</td>
<td>413</td>
<td>1352</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab34</td>
<td>7.06E-10 2.70E-08</td>
<td>N.B.</td>
<td>395</td>
<td>1770</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab35</td>
<td>2.21E-10 7.82E-09</td>
<td>6.44E-09</td>
<td>931</td>
<td>1391</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab36</td>
<td>4.65E-10 4.01E-09</td>
<td>N.B.</td>
<td>1258</td>
<td>1896</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab37</td>
<td>1.58E-09 P.F.</td>
<td>N.B.</td>
<td>1433</td>
<td>1434</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab38</td>
<td>9.60E-10 1.31E-08</td>
<td>N.B.</td>
<td>297</td>
<td>1832</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab39</td>
<td>2.13E-09 P.F.</td>
<td>6.41E-09</td>
<td>588</td>
<td>9029</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab40</td>
<td>1.97E-10 7.97E-09</td>
<td>6.03E-09</td>
<td>577</td>
<td>1452</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab41</td>
<td>2.14E-09 P.F.</td>
<td>8.64E-09</td>
<td>373</td>
<td>1017</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab42</td>
<td>4.03E-09 P.F.</td>
<td>N.B.</td>
<td>387</td>
<td>1234</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab43</td>
<td>4.37E-09 P.F.</td>
<td>N.B.</td>
<td>418</td>
<td>918</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab44</td>
<td>5.62E-10 9.46E-09</td>
<td>N.B.</td>
<td>1206</td>
<td>1249</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab46</td>
<td>4.45E-10 1.68E-08</td>
<td>2.46E-09</td>
<td>4257</td>
<td>1528</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab47</td>
<td>4.31E-10 7.37E-09</td>
<td>1.45E-09</td>
<td>10123</td>
<td>1833</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab48</td>
<td>3.50E-10 9.08E-09</td>
<td>1.41E-09</td>
<td>9770</td>
<td>1412</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab49</td>
<td>4.35E-10 1.74E-08</td>
<td>2.08E-09</td>
<td>4315</td>
<td>1392</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab50</td>
<td>7.29E-10 3.64E-08</td>
<td>N.B.</td>
<td>325</td>
<td>1435</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab51</td>
<td>2.84E-10 1.64E-09</td>
<td>N.B.</td>
<td>12077</td>
<td>1928</td>
<td>++</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Ab52</td>
<td>1.51E-09 5.75E-09</td>
<td>8.96E-11</td>
<td>15613</td>
<td>1411</td>
<td>+++</td>
<td>+++</td>
<td>ND</td>
</tr>
<tr>
<td>Ab53</td>
<td>6.35E-10 2.16E-08</td>
<td>N.B.</td>
<td>843</td>
<td>1338</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab54</td>
<td>5.00E-10 3.02E-09</td>
<td>N.B.</td>
<td>11001</td>
<td>1931</td>
<td>ND</td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td>Ab55</td>
<td>2.22E-09 9.17E-08</td>
<td>1.17E-09</td>
<td>6221</td>
<td>911</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab56</td>
<td>1.03E-09 5.18E-08</td>
<td>2.71E-09</td>
<td>745</td>
<td>1157</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab57</td>
<td>4.36E-10 1.83E-08</td>
<td>5.98E-10</td>
<td>10226</td>
<td>1227</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab58</td>
<td>5.75E-10 2.73E-09</td>
<td>N.B.</td>
<td>1870</td>
<td>1698</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab59</td>
<td>8.54E-10 5.72E-09</td>
<td>N.B.</td>
<td>3901</td>
<td>1754</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab60</td>
<td>5.59E-09 P.F.</td>
<td>P.F.</td>
<td>1871</td>
<td>741</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab61</td>
<td>4.61E-09 P.F.</td>
<td>N.B.</td>
<td>10517</td>
<td>1101</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab62</td>
<td>7.72E-10 1.42E-08</td>
<td>P.F.</td>
<td>4746</td>
<td>1647</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab63</td>
<td>1.13E-09 4.96E-08</td>
<td>N.B.</td>
<td>1722</td>
<td>1347</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab64</td>
<td>9.45E-10 6.57E-08</td>
<td>P.F.</td>
<td>8676</td>
<td>1458</td>
<td>+++</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Ab66</td>
<td>1.98E-09 6.07E-08</td>
<td>3.88E-09</td>
<td>4098</td>
<td>1161</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab67</td>
<td>6.60E-10 3.45E-08</td>
<td>2.92E-09</td>
<td>6207</td>
<td>1118</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab68</td>
<td>6.68E-09 1.09E-07</td>
<td>3.26E-09</td>
<td>2103</td>
<td>1135</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab69</td>
<td>3.75E-09 5.97E-08</td>
<td>3.40E-09</td>
<td>629</td>
<td>957</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab70</td>
<td>6.26E-09 P.F.</td>
<td>3.45E-09</td>
<td>3265</td>
<td>833</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab71</td>
<td>2.77E-09 6.47E-08</td>
<td>N.B.</td>
<td>804</td>
<td>1556</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab72</td>
<td>8.39E-10 2.94E-08</td>
<td>P.F.</td>
<td>3746</td>
<td>1500</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab73</td>
<td>4.10E-09 P.F.</td>
<td>N.B.</td>
<td>1117</td>
<td>1307</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab74</td>
<td>9.43E-09 P.F.</td>
<td>4.91E-09</td>
<td>1360</td>
<td>645</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab75</td>
<td>5.88E-09 P.F.</td>
<td>N.B.</td>
<td>276</td>
<td>676</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ab76</td>
<td>4.84E-09 P.F.</td>
<td>N.B.</td>
<td>1604</td>
<td>580</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Antibody</td>
<td>IgG $K_D$ hTREM2-Fc (M) Avid</td>
<td>IgG $K_D$ hTREM2-HIS (M) t</td>
<td>mTREM2 Cell binding MFI</td>
<td>hTREM2 Cell binding MFI</td>
<td>Phospho Syk hDC</td>
<td>Phospho Syk mDC</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>Ab77</td>
<td>P.F.</td>
<td>N.B.</td>
<td>1650</td>
<td>951</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab78</td>
<td>8.51E-10</td>
<td>3.73E-08</td>
<td>433</td>
<td>1435</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab79</td>
<td>1.07E-08</td>
<td>N.B.</td>
<td>738</td>
<td>900</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab80</td>
<td>5.73E-09</td>
<td>P.F.</td>
<td>8.85E-09</td>
<td>593</td>
<td>897</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab81</td>
<td>1.01E-08</td>
<td>P.F.</td>
<td>N.B.</td>
<td>400</td>
<td>994</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab82</td>
<td>1.10E-08</td>
<td>N.B.</td>
<td>5.82E-09</td>
<td>712</td>
<td>371</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab83</td>
<td>8.21E-09</td>
<td>N.B.</td>
<td>1576</td>
<td>684</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab84</td>
<td>1.16E-09</td>
<td>6.04E-08</td>
<td>N.B.</td>
<td>799</td>
<td>1148</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab85</td>
<td>5.72E-09</td>
<td>P.F.</td>
<td>2.19E-09</td>
<td>1121</td>
<td>460</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab86</td>
<td>3.89E-09</td>
<td>P.F.</td>
<td>6.55E-09</td>
<td>355</td>
<td>840</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ab87</td>
<td>9.51E-09</td>
<td>N.B.</td>
<td>4.48E-09</td>
<td>1228</td>
<td>414</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>88 control</td>
<td>No Binding</td>
<td>ND</td>
<td>ND</td>
<td>90.7</td>
<td>187</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>89 control</td>
<td>No Binding</td>
<td>ND</td>
<td>ND</td>
<td>142</td>
<td>185</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Luciferase Agonistic antibody activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab10</td>
<td>++++</td>
</tr>
<tr>
<td>Ab15</td>
<td>++++</td>
</tr>
<tr>
<td>Ab17</td>
<td>++++</td>
</tr>
<tr>
<td>Ab18</td>
<td>-</td>
</tr>
<tr>
<td>Ab20</td>
<td>+++</td>
</tr>
<tr>
<td>Ab24</td>
<td>-</td>
</tr>
<tr>
<td>Ab25</td>
<td>-</td>
</tr>
<tr>
<td>Ab28</td>
<td>-</td>
</tr>
<tr>
<td>Ab51</td>
<td>+++</td>
</tr>
<tr>
<td>Ab29</td>
<td>-</td>
</tr>
<tr>
<td>Ab54</td>
<td>+++</td>
</tr>
<tr>
<td>Ab64</td>
<td>+++</td>
</tr>
<tr>
<td>Ab66</td>
<td>+++</td>
</tr>
<tr>
<td>Ab89</td>
<td>-</td>
</tr>
<tr>
<td>Ab90</td>
<td>-</td>
</tr>
<tr>
<td>Isotype Control</td>
<td>-</td>
</tr>
<tr>
<td>PMA/Ion</td>
<td>+++</td>
</tr>
</tbody>
</table>

Example 42: Analysis of anti-stroke effect of agonistic TREM2 antibodies

[0525] Transient occlusion of the middle cerebral artery (MCAO) – a model that closely resembles human stroke is used to induce cerebral infarction in mice. Monofilament (70SPRe, Doccol Corp, USA) is introduced into the internal carotid artery through an incision of the right common carotid artery. The middle cerebral artery is occluded for 30 minutes with a range of reperfusion times (6 h, 12 h, 24 h, 2 d, 7 d and 28 d). The effect of surgery is controlled using
sham animals at 12 h and at 7 d. Sham animals undergo the same surgical procedure without occlusion of the middle cerebral artery. MCAO animals treated with agonistic anti-TREM2 antibodies or control antibodies are tested for infarct volumetry, acute inflammatory response (12 h reperfusion), transcription of pro-inflammatory cytokines TNFa, IL-1a, and IL-1b, microglial activity (CD68, Iba1), transcription of chemokines CCL2 (MCP1), CCL3 (MIP1a and the chemokine receptor CX3CR1 and invasion of CD3-positive T-cells (Sieber et al. (2013) PLoS ONE 8(1): e52982. doi:10.1371/journal.pone.0052982).

Example 43: Analysis of anti-Alzheimer’s disease effect of agonistic anti-TREM2 antibodies

To evaluate the ability of agonistic anti-TREM2 antibodies to delay, prevent, or reverse the development of Alzheimer’s disease (AD), 5X FAD mice are used. 5X FAD mice overexpress mutant human APP (695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) familial Alzheimer’s disease (FAD) mutations, along with human PS1 harboring two FAD mutations, M146L and L286V. Both transgenes are regulated by the mouse Thy1 promoter to drive overexpression on the brain and recapitulate major features of AD. Mice treated with the agonistic anti-TREM2 antibodies or with control antibodies are tested for A beta plaque load with immunohistochemistry and by ELISA of tissue extracts. They are further tested for the number of microglia in the brain, and for reduction in cognitive deficit using Morris Water maze, a spatial learning and memory task, Radial Arm Water Maze, a spatial learning and memory task, Y Maze (quantifies spontaneous alternation as a measure of spatial cognition), novelty preference in in an open field, operant learning to assess learning and memory, and fear conditioning (mousebiology.org website; Wang et al., (2015) Cell. pii: S0092-8674(15)00127-0).

Example 44: Analysis of the protective effect of antagonist TREM2 antibodies in respiratory tract infections

To evaluate the ability of antagonist TREM2 antibodies to delay, prevent, or treat bacterial respiratory tract infections, a preclinical mouse model involving challenge of C57Bl6 mice with *Streptococcus pneumoniae* is used. This model involves intranasal (i.n.) administration of 105 CFU *S. pneumoniae* serotype 3 (ATCC 6303) as described (see, e.g., Sharif O et al, 2014.

[0528] Ten to fifteen mice/group are challenged with *S. pneumoniae* and concomitantly are treated with antagonist anti-TREM2 antibodies every other day starting from day 0. The first dose of anti-TREM2 antibodies is administered 3 hours prior to challenge with *S. pneumonia*. Mice are monitored daily for 15 days to check for death events. % of mice surviving bacteria challenge is determined.

[0529] In separate experiments, count of bacterial load and cytokine expression in the blood and in the lungs is also determined. 24 or 48 hours after infection blood is collected in EDTA-containing tubes and plated on agar plates to enumerate bacterial CFU in the plasma. Plasma is stored at -20°C for cytokine analysis by ELISA. Lungs are harvested, homogenized and plated on agar plates to enumerate bacterial CFU, or incubated for 30 min in lysis buffer and supernatants analyzed for cytokine measurements.

[0530] In separate experiments, lungs are collected 40 hours post bacterial infection, fixed in 10% formalin, and embedded in paraffin for H&E pathology analysis.

Example 45: Analysis of the protective effect of antagonist TREM2 antibodies in sepsis

[0531] To evaluate the ability of antagonist TREM2 antibodies to delay, prevent, or treat sepsis, a preclinical mouse model involving systemic challenge of C57Bl6 mice with LPS is used. This model involves intraperitoneal (i.p.) administration of 37 mg/ml LPS as described (see, e.g., Gawish R et al, 2014 FASEB J). In this model >95% WT C57Bl6 mice succumb infection within 40 hours post LPS injection.

[0532] Cohorts of mice are challenged with LPS and concomitantly are treated with antagonist anti-TREM2 antibodies every day starting from day 0. The first dose of anti-TREM2 antibodies is administered 3 hours prior to challenge with LPS. Mice are monitored every ~4 hours during daytime, to check for death events. Percentage of mice surviving LPS challenge is determined.

[0533] In separate experiments, peritoneal lavage fluid (PLF) is collected. Supernatants are stored at -20°C for cytokine analysis by ELISA; pelleted cells are counted to quantify
inflammatory cells recruited in the peritoneal cavity. Similar studies can be conducted to test the efficacy of TREM2 antibodies in other models of infection (see, e.g., Sun et al., (2013) Invest Ophthalmol Vis Sci. 17:54(5):3451-62).

Example 46: Analysis of the protective effect of antagonist TREM2 antibodies in acute and chronic colitis

[0534] To evaluate the ability of antagonist anti-TREM2 antibodies to delay, prevent, or treat colitis, preclinical mouse models of acute or chronic colitis are used. For DSS-induced colitis, mice receive 3% DSS in drinking water ad libitum for 8 days. For TNBS-induced colitis, mice are anesthetized and treated with an intra-rectal injection of 3 mg TNBS in 20% ethanol (vol/vol) or vehicle alone as a control. For the chronic colitis model, all mice are treated with 3 cycles of 2% DSS for 5 days, followed by a 10-day recovery period. For all models, weight loss, stool consistency, and presence of fecal occult blood are monitored daily and used to calculate the disease activity index, as described (see, e.g., Correale C, 2013, Gastroenterology, February 2013, pp. 346-356.e3).

[0535] Cohorts of mice are treated with antagonist anti-TREM2 antibodies every day starting from day 0 and subjected to DSS or TNBS administration. Mice are monitored every day, to check for weight loss, stool consistency, and presence of fecal occult blood were monitored daily and used to calculate the disease activity index, as described (see, e.g., S. Vetrano, Gastroenterology, 135 (2008), pp. 173–184).

[0536] In separate experiments, endoscopic and histological images of mucosal damage are collected to evaluate inflammatory cell infiltration and mucosal damage. Similar studies can be conducted to test the benefit of TREM2 antibodies in other models of autoimmunity incuding Crohn's disease, inflammatory bowel disease, and ulcerative colitis (see, e.g., Low et al., (2013) Drug Des Devel Ther.; 7: 1341-1357; and Sollid et al., (2008) PLoS Med 5(9): e198).

Example 47: Analysis of the protective effect of agonist TREM2 antibodies in wound healing

[0537] To evaluate the ability of agonistic anti-TREM2 antibodies to increase colonic wound repair following injury, a mouse model of biopsy injury in the colon is used. In this model, the endoscope with outer operating sheath is inserted to the mid-descending colon and the mucosa is
surveyed to the ano-rectal junction. Then, a single full thickness area of the entire mucosa and submucosa is removed with flexible biopsy forceps with a diameter of 3 French, avoiding penetration of the muscularis propria. Each mouse is biopsy injured at 3–5 sites along the dorsal side of the colon (see, e.g., Seno H, 2008, *Proc Natl Acad Sci USA*. 2009 Jan 6; 106(1): 256-261).

[0538] Cohorts of mice are treated with agonist anti-TREM2 antibodies 2 or 3 days after biopsy injury. Mice are monitored every day for 15 days, to check for weight loss and wound healing by measuring the surface area of lesions.

Example 48: Analysis of the protective effect of antagonist TREM2 antibodies in retinal degeneration

[0539] Antagonist TREM2 antibodies decrease the accumulation and/or function of inflammatory macrophages, and as a result delay, prevent and/or treat age-related macular degeneration (AMD).

[0540] AMD is a degenerative disease of the outer retina. It is thought that inflammation, particularly inflammatory cytokines and macrophages, contribute to AMD disease progression.

[0541] The presence of macrophages in the proximity of AMD lesions is documented, in the drusen, Bruch’s membrane, choroid and retina. Macrophages release tissue factor (TF) and vascular endothelial growth factor (VEGF), which triggers the expansion of new blood vessels formation in patients showing choroidal neovascularization.

[0542] The type of macrophage present in the macular choroid changes with age, displaying elevated levels of M2 macrophages in older eyes compared to younger eyes. However, advanced AMD maculae had higher M1 to M2 rations compared to normal autopsied eyes of similar age. (see, e.g., Cao X et al. (2011), *Pathol Int* 61(9): pp528-35). This suggests a link between classical M1 macrophage activation in the eye in the late onset of AMD progression.

[0543] Retinal microglia cells are tissue-resident macrophages that are also normally present in the inner retina. In the event of damage, microglia can be activated and act as mediator of inflammation. Activated microglia has been detected in the AMD tissue samples and has been

[0544] Overall inflammatory macrophages (either M1 and/or activated microglia) are documented to correlate with AMD disease progression and therefore represent a therapeutic target for antagonist TREM2 antibodies. Similar therapeutic benefit can be achieved in glaucoma and genetic forms or retinal degeneration such as retinitis pigmentosa.


Example 49: Analysis of the protective effect of antagonist TREM2 antibodies in adipogenesis and diet-induced obesity

[0546] To test the effect of antagonist TREM2 antibodies in adipogenesis and obesity, a mouse model of high-fat diet (HFD) is used (see, e.g., Park et al., (2015) Diabetes. 64(1):117-27).

Example 50: Analysis of the protective effect of TREM2 antibodies in Malaria

[0547] TREM2 expression in the nonparenchymal liver cells closely correlates with resistance to liver stage infection with the malarial agent Plasmodium berghei (Gonçalves et al., (2013) Proc Natl Acad Sci 26;110(48):19531-6). Without wishing to be bound to theory, it is believed that TREM2 antibodies increase resistance to liver stage infection with P. berghei.

[0548] The ability of TREM2 antibodies to increase resistance to malaria infection is tested as described in Gonçalves et al., (2013) Proc Natl Acad Sci 26;110(48):19531-6. Briefly, GFP-
expressing *P. berghei* ANKA sporozoites are obtained by dissection of infected salivary glands from *Anopheles stephensi* mosquitoes. Sporozoite suspensions in RPMI medium are injected i.v. in 100 µL of inocula containing $10^4$ sporozoites per mouse. Livers are collected at 40 h after injection or survival, and parasitemia is followed for 28 days. For experimental cerebral malaria scoring, neurologic symptoms are monitored from day 5 after injection.

Example 51: Analysis of the protective effect of antagonist TREM2 antibodies in osteoporosis

[0549] Bone is a dynamic organ constantly remodeled to support calcium homeostasis and structural needs. The osteoclast is the cell responsible for removing both the organic and inorganic components of bone. The osteoclast is derived from hematopoietic progenitors in the macrophage lineage and differentiates in response to the tumor necrosis factor family cytokine receptor activators of NFκB ligand. Osteoclasts, the only bone-resorbing cells, are central to the pathogenesis of osteoporosis and osteopetrosis (Novack et al., 2008 *Annual Rev Pathol.*, 3:457-84).

[0550] Osteoporosis is a progressive bone disease that is characterized by a decrease in bone mass and density which can lead to an increased risk of fracture. It is mostly manifested in the first years following menopause, when bone turnover is accelerated, with increased activity of both osteoclasts and osteoblasts. Owing to an imbalance in the processes of resorption and synthesis, however, the net effect is bone loss, which is largely trabecular. Thus, the most prevalent sites of fracture in osteoporosis are the wrist, femoral neck, and vertebral bodies, in which the trabecular structure is key to overall bone strength. Accelerated osteoclast differentiation and increased bone resorption capacity, resulting in osteoporosis have been described in animal models lacking the expression of TREM2 (Otero et al (2012) *J. Immunol.*, 188, 2612–2621).

[0551] Reduced osteoclast function results in osteopetrosis, with increased bone mass and elimination of bone marrow space, as observed in animal models lacking DAP12 ITAM signaling adapter and resulting in a significant defect in differentiation of osteoclast-like cells (Koga, et al., (2004) *Nature* 428: 758–763).
Without wishing to be bound by theory, it is believed that administering an antagonist anti-TREM2 antibody of the present disclosure can prevent, reduce the risk of, and/or treat osteoporosis. In some embodiments, administering an agonist anti-TREM2 antibody may induce one or more TREM2 activities in an individual having osteopetrosis (e.g., DAP12 phosphorylation, Syk activation, and accelerated differentiation into osteoclasts) (Peng et al (2010). Sci Signal. 2010 18;3 122; and Humphrey et al., (2006) J Bone Miner Res., 21(2):237-45).
ANTIBODY VARIABLE REGION SEQUENCES

Ab1 – Heavy chain variable region (SEQ ID NO:242)
EVQLLESGGGLVQPGSRSLRLSCAASGFTFSSYAMSWVRQRPGKGEWVSLISNSGGSTY
YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA

Ab1 – Light chain variable region (SEQ ID NO:243)
EIVMTQSPATLSVSPGERATLSCRASQSVSLAWYQQPGAPRLLLYGA
RSFGSGSTTISLQSEDFAVYCCQLPYWPPFTFGGGTKVEIK

Ab2 – Heavy chain variable region (SEQ ID NO:244)
EVQLLESGGGLVQPGSRSLRLSCAASGFTFSSSAMSWVRQAPGKGEWVAS
YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK

Ab2 – Light chain variable region (SEQ ID NO:245)
EIVLTQSPATLSVSPGERATLSCRASQSVGSNLAWYQQKPGAPRLL
YSFGSGSTTISLQSEDFAVYCCQ

Ab3 – Heavy chain variable region (SEQ ID NO:246)
QVQLVQSGAEVKPGSSVKVSCKASGGTFTSSYAI
YAPFQGRVTITADESTAYMELSSLRSED

Ab3 – Light chain variable region (SEQ ID NO:247)
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLN
RSGSGSTTISLQPEDIA

sf-3499172 -227-
Ab4 – Heavy chain variable region (SEQ ID NO:248)
QVQLVQSGAEVKPGSSVKVSCKASGFTSFYIASWVRQAPGQGLEWMTGIIIPFGTAS
YAKKFQGRVTITADESTSYTAYMLELSSLRSEDTAVYYCeRCAGVDSIMDYWGQGTLVTVSS

Ab4 – Light chain variable region (SEQ ID NO:249)
EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLLYSASTRATGIIPAR
FSGSGSGTEFTLTISLQSEDFAVYYCQQDHDYPFTFGGGTKVEIK

Ab5 – Heavy chain variable region (SEQ ID NO:250)
QVQLVQSGAEVKPGASVKVSCKASGFTSYWYIHWVRQAPGQGLEWMIINPSSGST
SYAQKFQGRVTMTRDTSTSTVYMEELSSLRSEDTAVYYCARAPQESPYYVFDIWGQGMV
TVSS

Ab5 – Light chain variable region (SEQ ID NO:251)
EIVMTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQYFSSPFTFGGGTKVEIK

Ab6 – Heavy chain variable region (SEQ ID NO:252)
QVQLVQSGAEVKPGASVKVSCKASGFTSYWYIHWVRQAPGQGLEWMIINPSSGST
SYAQKFQGRVTMTRDTSTSTVYMEELSSLRSEDTAVYYCARGSPTYGYLYDPWGQGL
VTVSS

Ab6 – Light chain variable region (SEQ ID NO:253)
EIVLTQSPATLSVSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLYDASKRATGIPAR
FSGSGSGTDFTLTISLLEPEDFAVYYCQRVNLPPFTFGGTKVEIK
Ab7 – Heavy chain variable region (SEQ ID NO:254)
QVQLVQSGAEVKPGASVKSQCAASGTYFTFYMVHHWVRQAPGQGLEWMGGIGNPSGGSTTYAQKFGGRVIMTRDITSTSTIVYMELSSLRSEDATAVYYCARTSSKERDYWGQGTLVTVS

Ab7 – Light chain variable region (SEQ ID NO:255)
EIVLTQSPATLSLPGERATLSCRAQSQSYYSYLYWQQKPGQAPRLLIYDASKRGATGIPARFSGSGSGTDFLTTLTISSLEPDEDFAVYYCQQRISYPTFGGGKTVEIK

Ab8 – Heavy chain variable region (SEQ ID NO:256)
QLQLQESGPGLVKPSETLSLTCTVSGGSISSSYYYWGWIRQPPGKGLEWIGSISYSSTYYNPSSRSRVTISVDTSKQNFSKLSSSTVTADTAVYYCARGPYRLLGMDDVWQGTTVTVS

Ab8 – Light chain variable region (SEQ ID NO:257)
DIQMTQSPSSLSASVGVDRVITITCRASQSISSLNYWQQKPGKAPKLLIYGAQSSLQSGVPSRFSGSGSGTDFLTLTISSLQPEDFATYCCQIDTDTPFGGGKTVEIK

Ab9 – Heavy chain variable region (SEQ ID NO:258)
EVQLVQSGAEVKPGESLKSCKGSYGFTSYWGWVQMRPGKGLEWMGIIPGPDSSTTYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCRLHISGEVNWFDWQGTLVT

Ab9 – Light chain variable region (SEQ ID NO:259)
EIVLTQSPATLSLPGERATLSCRAQSQSYYSYLYWQQKPGQAPRLLIYDASRNATGIPARFSGSGSGTDFLTTLTISSLEPDEDFAVYYCQFYSYPWTPFGGGKTVEIK
Ab10 – Heavy chain variable region (SEQ ID NO:260)
EVQLVQSGAEVKPGESLKISCKGSYSFTSNWIGWVRQMPGKLEWMGIIYPGDSDTVYSFPGQVTISADKSIISTAYLQWSSLKASDTAMYCAREAGYDYGELAFDIWGGQTMTTVSS

Ab10 – Light chain variable region (SEQ ID NO:261)
EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAASSRATGIPDRFSGSGSTDTFLTTLTISRLEPEDFAVYYCQHQDSPPFTFGGGTKVEIK

Ab11 – Heavy chain variable region (SEQ ID NO:263)
EVQLVQSGAEVKPGESLKISCKGSYSFTTYWIGWVRQMPGKLEWMGIIYPGDSDTVYSFPGQQVTISADKSIISTAYLQWSSLKASDTAMYCARAGHYDGGHLMGDVMVGQGTMTTVSS

Ab11 – Light chain variable region (SEQ ID NO:264)
EIVLTQSPGTLSSLSPGERATLSCRASQSVSSDYLAWYQQKPGQAPRLLIYGAASSRATGIPDRFSGSGSTDTFLTTLTISRLEPEDFAVYYCQQDYPWTFGGGTKVEIK

Ab12 – Heavy chain variable region (SEQ ID NO:264)
EVQLVQSGAEVKPGESLKISCKGSYSFTSYWIGWVRQMPGKLEWMGIIYPGDSDTVYSFPGQQTISADKSIISTAYLQWSSLKASDTAMYCARLGHYSGTVSSYGMDVMVGQGTMTTVSS

Ab12 – Light chain variable region (SEQ ID NO:265)
DIQMTQSPSSLSASVGRVTITCRASQSISSYNWYQQKPGKAPKLIIYAASSLSGVPSSRFSGSGSTDTFLTISLQPEDFATYYCQQEYAVPYTFGGGTKVEIK
Ab13 – Heavy chain variable region (SEQ ID NO:266)
QVQLVQSGAEVKPGASVVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWASAYNGNTNYAQKLGQRVTMDTSTAYMLERSLRSDDTAVYVCARGPSHYDIALWGGQGTLVTVSS

Ab13 – Light chain variable region (SEQ ID NO:267)
EIVLTQSPATLSLSPGERATLSCRAQSQSYYLAWYYQKPGQAPRLYLDASNREATGIPARFSGSGSGTDFTTLISSLEPEDFAVYCQVSNYPIFFGGLGTKVEIK

Ab14 – Heavy chain variable region (SEQ ID NO:268)
QVQLQESGPGLVKPSQTLSTCTVSGSISASSGGYWSWGHRQHPKGLAWIGNYYSGSTYVNPSSLRVTISVTDSTKNQFSQLKSSLSTMATAADTAVYVCARGLYGYGVLDVWGQGTMVTVSS

Ab14 – Light chain variable region (SEQ ID NO:269)
DIQMTQSPSSLSASVGDRVTITCQASQDI5NYLNYWYQQKPGKAPKLLYDASNLETGVPSRSGSGSGTDFTFTTLSSLQPEPATAYCQVQVDNIPPTFGGLGTKVEIK

Ab15 – Heavy chain variable region (SEQ ID NO:268)
QVQLQESGPGLVKPSQTLSTCTVSGSISASSGGYWSWGHRQHPKGLAWIGNYYSGSTYVNPSSLRVTISVTDSTKNQFSQLKSSLSTMATAADTAVYVCARGLYGYGVLDVWGQGTMVTVSS

Ab15 – Light chain variable region (SEQ ID NO:270)
DIQMTQSPSSLSASVGDRVTITCQASQDI5NYLNYWYQQKPGKAPKLLYDASNLETGVPSRSGSGSGTDFTFTTLSSLQPEPATAYCQVQFDTYPPTFGGLGTKVEIK
Ab16 – Heavy chain variable region (SEQ ID NO:271)
QLQLQESGPGLVKPSETLSLTCTVSGGSISSNSYWWGYWIRQPPGKGLEWIGSIYYSGSTYY
NPSLKRVTISVDTSNKQFSLKLSSVTAADTAVYCARGVGLGYVFYWGQGTLVTVSS

Ab16 – Light chain variable region (SEQ ID NO:272)
DIQMTQSPSSLSASVGVDRVTITCQASQDISNLYNWYQQPKPGKAPKLIIYDASNLETGVPS
RFSGSGSTDFTFTISSLQPEDIATYYCQQFNLFTFGGKTVEIK

Ab17 – Heavy chain variable region (SEQ ID NO:271)
QLQLQESGPGLVKPSETLSLTCTVSGGSISSNSYWWGYWIRQPPGKGLEWIGSIYYSGSTYY
NPSLKRVTISVDTSNKQFSLKLSSVTAADTAVYCARGVGLGYVFYWGQGTLVTVSS

Ab17 – Light chain variable region (SEQ ID NO:273)
DIQMTQSPSSLSASVGVDRVTITCQASQDISNLYNWYQQPKPGKAPKLIIYDASNLETGVPS
RFSGSGSTDFTFTISSLQPEDIATYYCQQFNLFTFGGKTVEIK

Ab18 – Heavy chain variable region (SEQ ID NO:274)
QVQLQESGPGLVKPSETLSLTCTVSGSISYSYYWSWIRQPPGKGLEWIGSIYYSGSTYNNP
SLKSRVTISVDTSNKQFSLKLSSVTAADTAVYCARDGGYEPYSPGTPFDIWGQGTMVTVSS

Ab18 – Light chain variable region (SEQ ID NO:275)
DIQMTQSPSSLSASVGVDRVTITCQASQDISNLYNWYQQPKPGKAPKLIIYDASNLETGVPS
RFSGSGSTDFTFTISSLQPEDIATYYCQQFIDLPFTFGGKTVEIK

Ab19 – Heavy chain variable region (SEQ ID NO:274)
QVQLQESGPGLVKPSETLSLTCTVSGSISYYWSWIRQPPGKGLEWIGSIYYSGSTYNNP
SLKSRVTISVDTSNKQFSLKLSSVTAADTAVYCARDGGYEPYSPGTPFDIWGQGTMVTVSS
Ab19 – Light chain variable region (SEQ ID NO:276)
DIQMTQSPSSLSASVGVPRPITCQASQDISNYLNWYQQKPGKAPKLLYDASNLETGVPS
RFSGSGSTDFDTFTISSLQPEDIATYYCQYYDLPFTFGGGTKVEIK

Ab20 – Heavy chain variable region (SEQ ID NO:277)
QVQLQESGPGLVKPSETLALTCTVSGGSISSYWSWIRQPPGKGLEWIGSIYYSGSTYNLP
SLKSRVTISVDTSKNNQFSLKLSTVTAAADTAAYYCARSGMASFFDYWGGQTLVTVSS

Ab20 – Light chain variable region (SEQ ID NO:278)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSDYLAWYQQKPGQAPRLLNYGASSRATEIP
DGRFSGSGSTDFTDLTDTRLSELEPEDFAVYCYQFSSHPFTFGGGTKVEIK

Ab21 – Heavy chain variable region (SEQ ID NO:410)
EVQLVQSGAEVKPGESLKLISCKGSGYSFTTVIGWVRQMPGKGLEWMGIYIPGDSDTTR
YSFQGQVTISADKSISTAYLQWSSLKASDTAMYCARAGHYDGGHGLMDVWGQGT
TVTVSS

Ab21 – Light chain variable region (SEQ ID NO:411)
EIVMTQSPGTLSLSPGERATLSCRASQSVSWSYLYWYQQKPGQAPRLLNYGASNATGIP
DGRFSGSGSTDFTDLTDTRLSELEPEDFAVYCYQDAPYTFGGGTKVEIK

Ab22 – Heavy chain variable region (SEQ ID NO:262)
EVQLVQSGAEVKPGESLKISCKGSGYSFTTVIGWVRQMPGKGLEWMGIYIPGDSDTTR
YSFQGQVTISADKSISTAYLQWSSLKASDTAMYCARAGHYDGGHGLMDVWGQGT
TVTVSS

Ab22 – Light chain variable region (SEQ ID NO:279)
EIVMTQSPGTLSLSPGERATLSCRASQSVSWSYLYWYQQKPGQAPRLLNYGASNATGIP
DGRFSGSGSTDFTDLTDTRLSELEPEDFAVYCYQDAPYTFGGGTKVEIK
Ab23 – Heavy chain variable region (SEQ ID NO:280)
EVQLLESGGGLVQPGGLRLSCAASGFTSSYAMSWVRQAPGKGLEWVSAISGSSTY
YADSVKGRFTISRDNKNTLYLMQNSLRAEDTAVYYCAKLGHSMDVWGQGTTTVTS
S

Ab23 – Light chain variable region (SEQ ID NO:281)
DIVMTQSPDSLAVSLGERATINCCKSSQSVLYSSNNKNYLAWYQQKPGQPKLLISWASTR
ESGVPDRFSGSGTDLTISLQAEDVAVVYQQAYLPPITFGGGTKEIK

Ab24 – Heavy chain variable region (SEQ ID NO:282)
EVQLLESGGGLVQPGGLRLSCAASGFTSSYAMSWVRQAPGKGLEWVSAISGSSTY
YADSVKGRFTISRDNKNTLYLMQNSLRAEDTAVYYCAKPLKRGGRGFYWQGTLVTVS
S

Ab24 – Light chain variable region (SEQ ID NO:283)
DIQMTQSPSSLSASVGDRVVTITCRASQSISSYLNYWYQQKPGKAPKLLIYAASSLQSGVPSR
FSGSGSGTDLTISLQPEDFYTYCQQAFSPPPWTFGGGTKEIK

Ab25 – Heavy chain variable region (SEQ ID NO:284)
EVQLLESGGGLVQPGGLRLSCAASGFTSSYAMSWVRQAPGKGLEWVSVISGSSTY
YADSVKGRFTISRDNKNTLYLMQNSLRAEDTAVYYCAKEGRTTMDWGQGTLVTVSS

Ab25 – Light chain variable region (SEQ ID NO:285)
EIVLTQSPGTLSSPSGERATLSCRASQSVSSSLNYWYQQKPGQAPRLLYGAAGSRATGIDP
RFSGSQSGTDFTLISRLPEPDFAVYYCQQDPPSTFGGGTKEIK

Ab26 – Heavy chain variable region (SEQ ID NO:286)
EVQLLESGGGLVQPGGLRLSCAASGFTSSYAMSWVRQAPGKGLEWVSVISGSSTY
YADSVKGRFTISRDNKNTLYLMQNSLRAEDTAVYYCAKQDSVQLDYWGQGTLVTVSS
Ab26 – Light chain variable region (SEQ ID NO:287)
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFLTISLEPEDFAVYYCQQEFDFLPFTFGGGTKVEIK

Ab27 – Heavy chain variable region (SEQ ID NO:288)
EVQLLESGGGLVQPGGLRLSCLAASGFTFSSYAMSWVRQPQKGLEWVSAISGSGGSTY
YADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAK KYSSRGVYFYF DYWGQ GTLV
TVSS

Ab27 – Light chain variable region (SEQ ID NO:289)
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFLTISLEPEDFAVYYCQQYNNFPPTFGGGTKVEIK

Ab28 – Heavy chain variable region (SEQ ID NO:290)
EVQLLESGGGLVQPGGLRLSCLAASGFTFSSYAMSWVRQPQKGLEWVSAISGSGGSTY
YADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARLGGAVGARHVTYFDYWGQ
GTLVT TVSS

Ab28 – Light chain variable region (SEQ ID NO:291)
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLIIYDASKRATGIPAR
FSGSGSGTDFLTISLEPEDFAVYYCQQRYLRPITFGGGTKVEIK

Ab29 – Heavy chain variable region (SEQ ID NO:292)
QVQLVESGGGVQPGRSLSCAASGFTFSSYGMHWVRQAPGGKGLEWVAVISYDGSNK
YYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARQYYGGSGWDPWGQQT
LVTVSS

Ab29 – Light chain variable region (SEQ ID NO:293)
EIVLTQSPGTLSSLPGERATLSCRASQSVSSYLSLAWYQQKPGQAPRLIIYGASSRATGIPD
RFSGSGSGTDFLTISRLEPEDFAVYYCQQPAGVPTFGGGTKVEIK

sf-3499172 -235-
Ab30 – Heavy chain variable region (SEQ ID NO:294)
EVQLLESGGGLVQPGGLRLSCAASGFTSSYSAMSWVRQAPGKGLEWVSAISGSGGYT
YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLQQEYAYFQHWGGTLV
T
VSS

Ab30 – Light chain variable region (SEQ ID NO:295)
DIQMTQSPSSLSASVGDRVITCRASQSISSYLNWYQQKPGKAPKLLIYGASSLQSGVPS
RFSGSGLGTDFLTISSLQPEDFAYYYCQQVYITPTITFGGGTKVEIK

Ab31 – Heavy chain variable region (SEQ ID NO:297)
QVQLVESGGGVVQPSRSLRLSCAASGFTSSYGMHWVRQAPGKGLEWVALIYWDGSN
KYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARRRDGYYDEVFDIWGQGT
MVT
VSS

Ab31 – Light chain variable region (SEQ ID NO:297)
DIQLTQSPSSLASVGDRVITTCQASQDISNFLNWYQQKPGKAPKLLIYDASNLETGVPS
RFSGSGLGTDFLTISSLQPEDIATYYCQQPVDLPFTFGGGTKVEIK

Ab32 – Heavy chain variable region (SEQ ID NO:298)
EVQLLESGGGLVQPGGLRLSCAASGFTSSYSAMSWVRQAPGKGLEWVSAISGSGGYT
YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVPKHYYVVLWDYGQGT
LV
T
VSS

Ab32 – Light chain variable region (SEQ ID NO:299)
EIVLTQSPATLSGGERATLSRASQSVSSYLAQWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGLGTDFLTISSLQPEDFAVYYCQYSSFPPTFGGGTKVEIK
Ab33 – Heavy chain variable region (SEQ ID NO:300)
QVQLVESGGGVQPGGLRLSCAASGFTFSSYGMHWVRQAPGKGLRWVAVISYDGSKNYYADSVKGRTISRDNSKNTLYLQMNSLRAEDTAVYYCARAGGHLEDFYWGQGTLVTVSS

Ab33 – Light chain variable region (SEQ ID NO:301)
EIVLTQSPATLSLSPGERATLSCRASQSVSYYLAWYQQKPGQAPRLILYIDASNRATGIPARFSGSGSTDFTLTISSLREPEDFAYYCQQQDFSSFPFPGGGTKEIK

Ab34 – Heavy chain variable region (SEQ ID NO:302)
QVQLVESGGGVQPGGLRLSCAASGFTFSSYGMHWVRQAPGKGLRWVAVISYDGSKNYYADSVKGRTISRDNSKNTLYLQMNSLRAEDTAVYYCARGDRGGEVDFDIFWGGQGETTVSS

Ab34 – Light chain variable region (SEQ ID NO:303)
DIQMTPSPLLSASVGDRVTITCRASQSISSYLNWyQQKPGKAPKLIIYAAAASSLQSGVPSRFSGSGSTDFTLTISSLQPEDFATYCCQQQDFSSFPFPGGGTKEIK

Ab35 – Heavy chain variable region (SEQ ID NO:304)
EVQLLESGGLVQPGGLRLSCAASGFTFSSYAMESWVRQAPGKGLRWVSAISGSGSTYHYADSVKGRTISRDNSKNTLYLQMNSLRAEDTAVYYCARTRSGYVQRNYFDYWGQGTLVTVSS

Ab35 – Light chain variable region (SEQ ID NO:305)
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWyQQKPGKAPKLIIYAAAASSLQSGVPSRFSGSGSTDFTLTISSLQPEDFATYCCQQQGGYSAFITFPGGGTKEIK

sf-3499172 -237-
Ab36 – Heavy chain variable region (SEQ ID NO:306)
QVQLVESGGGVQPGSRSLRLSCAASGFITFSTYGHMHWVRQAPGKGLEWVAVIWyDGSN
KYYADSVKGRFTISRDSKNTLQLQMNSLRAEDTAHYYCARGTGAAASPAFDIWGQG
TMVTSS

Ab36 – Light chain variable region (SEQ ID NO:307)
EIVLTQSPATLSLPGERATLSCRASQSVSSYLVawyQQKPGQAPRLIIYDASNRTGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYCYQQLFDWPTFGGGTKVEIK

Ab37 – Heavy chain variable region (SEQ ID NO:308)
EVQLLESGGGLVQPGSLRLSCLRASQSVSSYLYMWSVRQAPGKGLEWVASISGSGGSTY
YADSVKGRFTISRDSKNTLQLQMNLSRAEDTAHYYCARGTGYMLDMGVGWGTAV
TVSS

Ab37 – Light chain variable region (SEQ ID NO:309)
EIVLTQSPATLSLPGERATLSCRASQSVSSYLVawyQQKPGQAPRLIIYDASNRTGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYCYQQRAFLFTFGGGTKVEIK

Ab38 – Heavy chain variable region (SEQ ID NO:310)
QVQLVESGGGVQPGSRSLRLSCLRASQSVSSYLMHWVRQAPGKGLEWVAVIWyDGSN
KYYADSVKGRFTISRDSKNTLQLQMNLSRAEDTAHYYCARGAPVDYGGIEPEYFQHW
GQGTLVTSS

Ab38 – Light chain variable region (SEQ ID NO:311)
EIVLTQSPATLSLPGERATLSCRASQSVSSYLVawyQQKPGQAPRLIIYDASNRTGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYCYQQIDFLPYTFGGGTKVEIK
Ab39 – Heavy chain variable region (SEQ ID NO:312)
EVQLLESGGGLVQPGGLRLSCAASGFTSFSSYAMSWVRQAPGKGLEWSAISGSGSTY
YADSVKGRFTISRDSKNTLQLQMNSLRAYEDTAAYVYYCAKHGYHVGIADFIWGQGMVT
SS

Ab39 – Light chain variable region (SEQ ID NO:313)
DIQMTQSPSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLIIYAAASLQSGVPSR
FSGSGTDTTLTISSLQPEDFAYYCQVYSPPIFTGGGTKVEIK

Ab40 – Heavy chain variable region (SEQ ID NO:304)
EVQLLESGGGLVQPGGLRLSCAASGFTSFSSYAMSWVRQAPGKGLEWSAISGSGSTY
YADSVKGRFTISRDSKNTLQLQMNSLRAYEDTAAYVYYCARTRSGYGDNYFDYGWGQT
LVTSS

Ab40 – Light chain variable region (SEQ ID NO:314)
DIQMTQSPSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLIIYAAASLQSGVPSR
FSGSGTDTTLTISSLQPEDFAYYCQVYSPPIFTGGGTKVEIK

Ab41 – Heavy chain variable region (SEQ ID NO:315)
EVQLLESGGGLVQPGGLRLSCAASGFTSFSTYAMSWVRQAPGKGLEWSAISGSGSTY
YADSVKGRFTISRDSKNTLQLQMNSLRAYEDTAAYYCARAKRAMKSVAFDIWGQMT
VTVSS

Ab41 – Light chain variable region (SEQ ID NO:316)
EIVLTQSPATLSPLSRGERATSLSCRASQSYLVAYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGTDTTLTISSLQEDFVTYYCQYRALPITFGGTKVEIK
Ab42 – Heavy chain variable region (SEQ ID NO:317)
EVQLLESGGGLVQPGGLRLSCAASGFTSFSSAMSWVRQAPGKGLEWVSAISGSGGGSTY
YADSVVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVPSYQRGTAFDPWGGTLV
TVSS

Ab42 – Light chain variable region (SEQ ID NO:318)
EIVLTQSPGTLSLSPGERATLSRASQSVSSYLYAWYQQKPGQAPRLLIYGASSRATGIPD
RSFSGSGTDFTLTISSLLEPEDFAYVYCQGYSPTFPGGGTKVEIK

Ab43 – Heavy chain variable region (SEQ ID NO:319)
EVQLLESGGGLVQPGGLRLSCAASGFTSFSSAMSWVRQAPGKGLEWVSAISGSGGGSTY
YADSVVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSPAVAGIYRADYWGQGTLY
TVSS

Ab43 – Light chain variable region (SEQ ID NO:320)
DIQMTQSPSSLSASVGDRVTITCRASQSIYRNYWIIQQKPGKAPKLILYAASSLQSGVPSR
FSQSGSGTDFTLTISSLLEPEDFATYYCQVYMYSTYPSFFDGKEIK

Ab44 – Heavy chain variable region (SEQ ID NO:306)
QVQLVESGGGVVQPGRRSLCASAGFTSTYGMMHWVRQAPGKGLEWVAVIWDGSN
KYYADSVVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGTGAASPAFDIWGQG
TMVTVSS

Ab44 – Light chain variable region (SEQ ID NO:321)
EIVMTQSPATLSLSPGERATLSRASQSVSSYLYAWYQQKPGQAPRLLIYDSNRATIGIPAR
FSQSGSGTDFTLTISSLLEPEDFAYVYCQLVHWPTFPGGGTKVEIK
Ab45 – Heavy chain variable region (SEQ ID NO:322)
QVQLVQSGAEVKPGASVVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMIINPSGGS
TSYAAQKFGQVRVTMTRDTSTSTVYMELSSLRSEDATAVYYCARPGYTTALDDYYYMDVVGKGTTVTSS

Ab45 – Light chain variable region (SEQ ID NO:323)
EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPA
RFSGSGSGTEFTLTISLQSEDFAVYYCQLDDWFTFGGGTKVEIK

Ab46 – Heavy chain variable region (SEQ ID NO:324)
QVQLVQSGAEVKPGASVVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMIINPSGGS
TSYAAQKFGQVRVTMTRDTSTSTVYMELSSLRSEDATAVYYCARPAKTADYWGQGTLVTVSS

Ab46 – Light chain variable region (SEQ ID NO:325)
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDSSNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQRSNYPITFGGGTKVEIK

Ab47 – Heavy chain variable region (SEQ ID NO:326)
QVQLVQSGAEVKPGASVVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMIINPSGGS
TTYAQKFGQVRVTMTRDTSTSTVYMELSSLRSEDATAVYYCARPGKSMDVWGQGTTVTSS

Ab47 – Light chain variable region (SEQ ID NO:327)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQRILYPITFGGGTKVEIK
Ab48 – Heavy chain variable region (SEQ ID NO:326)
QVQLVQSGAEVKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGS
TTYAQQKFGQGRVTMTRDTSTSTVYMELESSLRSEDTAVYYCARPGKSMDVWGQGTITVTVSS

Ab48 – Light chain variable region (SEQ ID NO:328)
EIVLTQSPATLSLPGERATLSCRASQSVSSYLYAWYQQKPGQPRLIIYDASNRATGIPAR
FSGSNGTDFTLTISSLEPEDFAVYYCQQQRAAAYPIIFGGGTKVEIK

Ab49 – Heavy chain variable region (SEQ ID NO:324)
QVQLVQSGAEVKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGS
TSYAQQKFGQGRVTMTRDTSTSTVYMELESSLRSEDTAVYYCARPAKTADYWQGTLVTVS
S

Ab49 – Light chain variable region (SEQ ID NO:329)
EIVLTQSPATLSLPGERATLSCRASQSVSSYLYAWYQQKPGQPRLIIYDASKRATGIPAR
FSGSNGTDFTLTISSLEPEDFAVYYCQYRQRTSHPITFGGGGTKVEIK

Ab50 – Heavy chain variable region (SEQ ID NO:250)
QVQLVQSGAEVKPGASVKVSCKASGYTFTSYIHYWVRQAPGQGLEWMGIINPSGGST
SYAQQKFGQGRVTMTRDTSTSTVYMELESSLRSEDTAVYYCARAPQESPYPFVDFIWGQGTMV
TVSS

Ab50 – Light chain variable region (SEQ ID NO:330)
EIVLTQSPGTLSLPGERATLSCRASQSVSSYLYAWYQQKPGQPRLIIYGASSRATGIPD
RFSGSNGTDFTLTISSLEPEDFAVYYCQQYAGSPFTFGGGGTKVEIK
Ab51 – Heavy chain variable region (SEQ ID NO:331)
QVQLVQSGAEVKPGASVKSQCKASGYTFTSYYMHWRQAPGKGLEWMGIINPSGGTSYAQKFWQRVTMTRDTSTSTVYMELSLRSEDTAIVYCARGVGGSQDYYYMDVWGKGTTVTVSS

Ab51 – Light chain variable region (SEQ ID NO:332)
DIQMTQSPSSLSASVGVFTITCRASQSISSYLNWYQQKPGKAPKLIIYAASSLQSGVPSRFSGSNGTDFTLTISSLQPEDFATYYCQQFDDVFITGSGGTKEIK

Ab52 – Heavy chain variable region (SEQ ID NO:412)
QVQLVQSGAEVKPGASVKSQCKASGYTFTSYYIHVRQAPGKGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSLRSEDTAIVYCARGDDSSGYPLGLDVGWQGTMVTVSS

Ab52 – Light chain variable region (SEQ ID NO:413)
EIIVMTQSPATLSVSPGERATLSCRASQSVSSNALWYQQKPGQPRLLIYGAISTRATGIPARFSGSNGTEFTLTISSLQSEDFAVYYCQQVNSLPPFTGGGTKVEIK

Ab53 – Heavy chain variable region (SEQ ID NO:250)
QVQLVQSGAEVKPGASVKSQCKASGYTFTSYYIHVRQAPGKGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSLRSEDTAIVYCARAPQESPYYFDIWGQGTMOVTVSS

Ab53 – Light chain variable region (SEQ ID NO:333)
EIIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQPRLLIYGAISTRATGIPDPFSGSNGTDFTLTIISRLEPEDFAVYYCQQVNSLPPFTGGGTKVEIK

sf-3499172 -243-
Ab54 – Heavy chain variable region (SEQ ID NO:322)
QVQLVQSGAEVKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAYYCARGPGYTALDYYYMDWVGKGTTVVSS

Ab54 – Light chain variable region (SEQ ID NO:334)
DIQMTQSPSSLSAVGVDRVTITCRASQSINSYLNWYQQKPGKAPKLIYAAASLQSGVPSRFSGSGSGTDTFLTIISSLQPEDFATYYCQQSDDDPFTFGGTKEIK

Ab55 – Heavy chain variable region (SEQ ID NO:335)
QVQLVQSGAEVKPGASVKVSCKASGYTFTGSYMHWVRQAPGQGLEWMGWINPSGSGTNYAQKFQGRVTMTRDTSTAYMELSRLSDDTAAYYCARGPLYHPMIDYWQGTLVTVSS

Ab55 – Light chain variable region (SEQ ID NO:336)
EIVLTQSPATLSLSPGERALTSCRASQSVESYLAWYQQKPGQAPRLLIIYDASNRTGIPARFSGSGSGTDTFLTIISSLLEPEDFAVYYCQLSTYPTFGGTKEIK

Ab56 – Heavy chain variable region (SEQ ID NO:337)
QVQLVQSGAEVKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMSGNPNSGSGTNYAQKFQGRVTMTRDTSTAYMELSRLSDDTAAYYCARRASSVNDWGQGTLVTVSS

Ab56 – Light chain variable region (SEQ ID NO:338)
EIVMVTQSPATLSLSPGERALTSCRASQSVESYLAWYQQKPGQAPRLLIIYDASNRTGIPAIFESTGSGTDTFLTIISSLLEPEDFAVYYCQQRSVYPTFGGTKEIK
Ab57 – Heavy chain variable region (SEQ ID NO:339)
QVQLVQSGAEVKPGASVKVSCKASGYTFTNYGSRWRQAPGQGLEWMGWISAYNGNTNYAQLQGRVTMTTDISTSTAYMELRSLRSDTFAVYYCARGPRTKAYYGSGYVVFDPWGQGTTVSS

Ab57 – Light chain variable region (SEQ ID NO:340)
EIVLTQSPATLSLSPGERATLASCRASQSVSSYLAWYYQQKPGQPRLIIYDASKRATGUIPARFSGSQGTDFTLTISSLEPEDFAVYYCQVSLFPLTFTGGKTVEIK

Ab58 – Heavy chain variable region (SEQ ID NO:341)
EVQLVQSGAEVKPGESLKISCKGSYSFTSYWIGWVRQAPGKGLEWMGIIPGDSDTTRYSPSFQGVVTISADKSISTAYLQWSSLKASDTAMYCARLGTYSTGATAFDIWGQGTMVTVSS

Ab58 – Light chain variable region (SEQ ID NO:342)
DIQMTQSPSLASVGDRVTITCRASQSISSLWALWAYQQKPGKPKLIYDASSLESGVPSRFSGSGTTEFTLTISSLQPDDFATYYYCLDNSYNTFGGGTKEIK

Ab59 – Heavy chain variable region (SEQ ID NO:343)
QVQLVQSGAEVKPGASVKVSCKASGYTFTGSYMHVRQAPGQGLEWMGWINPNGGTNYAQQKFGQGRVTMTRDTSISTAYMELRSLRSDTFAVYYCARGGNYVYSLFDFGWGQTMTVSS

Ab59 – Light chain variable region (SEQ ID NO:344)
DIQMTQSPSLASVGDRVTITCQASQDISNYLNWYYQQKPGKAKPLKIYDASNLSTGVPSRFSGSGTDFDFTISSLQPEDATYYCQHIALPFITFGGGTKEIK
Ab60 – Heavy chain variable region (SEQ ID NO:345)
QVQLVQSGAEVKPGASVKVSCKASQYFTGNYMHWVRQAPGQGLEWMGWNSG
FTSYAQQKFGGRVTMTRDDTSTAYMELESRLRSDDTAVVYCARNASKMGDDWGQGTLVT
VSS

Ab60 – Light chain variable region (SEQ ID NO:346)
EIVLTQSPATLSLSPGERATLSRASQSVSSYLAWYQQKPGQAPRLLIYDASKRATGIPAR
FSGSGSTDFTLTISSLEPEDFAVYYCQQPMSPITFGGGTKEIK

Ab61 – Heavy chain variable region (SEQ ID NO:347)
QVQLVQSGAEVKPGASVKVSCKASQYFTFSGNYMHWVRQAPGQGLEWMGWISAYNGN
TNYAQQKLGQRVTMTTDTSTAYMELESRLRSDDTAVVYCARGVPRVSYFQHWGQGT
LVTVSS

Ab61 – Light chain variable region (SEQ ID NO:348)
EIVLTQSPATLSLSPGERATLSRASQSVSSYLAWYQQKPGQAPRLLIYDSSNRATGIPAR
FSGSGSTDFTLTISSLEPEDFAVYYCQQAFNRPPFTFGGGTKEIK

Ab62 – Heavy chain variable region (SEQ ID NO:349)
EVQLVQSGAEVKPGSNSLRKICSGSDFHYWIGWVRQMPGKGLEWMGIYPPGDSRT
YSPSFQGQVTISADKSIStAYLQWSSLKASDTAMYCARAGHYDDWSGLGLDVWQGT
MVTVSS

Ab62 – Light chain variable region (SEQ ID NO:350)
EIVLTQSPATLSLSPGERATLSRASQSVSSYLAWYQQKPGQAPRLLIYDASKRATGIPAR
FSGSGSTDFTLTISSLEPEDFAVYYCQQSVHPYTFGGGTKEIK

sf-3499172

-246-
Ab63 – Heavy chain variable region (SEQ ID NO:351)
QVQLVQSGAEVKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWGMGWTSTYNQ
TYAQLKQGRVTMTTDTSTAYMELRSLRSDDTAIVYCCARGSGGYDSWYDQGT
LVTVSS

Ab63 – Light chain variable region (SEQ ID NO:352)
DIQMTQSPSSASVGDRVVTITCRASQGIDSLAWYQQKPGKAPKLLIYAASSLQSGVPS
RFSGSGSGTDFTLTISLQPEDFATYYCQQAYSLPFTFQGGTKEIK

Ab64 – Heavy chain variable region (SEQ ID NO:353)
EVQLVQSGAEVKKPGESLKISCKGSYGSTDFTYWIGWVRQMPGKLEWGMGIIPGDSDTR
YSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYCARLGRWSSGTAFDIWWQGTMV
TVSS

Ab64 – Light chain variable region (SEQ ID NO:354)
EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQPRLSIIYGASTRATGIPA
RFSGSGSGTEFTLTISLQSEDFAVYYCQDQDDGYTFQGGTKEIK

Ab65 – Heavy chain variable region (SEQ ID NO:355)
EVQLVQSGAEVKKPGESLKISCKGSYGSTDFTYWIGWVRQMPGKLEWGMGIIPGDSDTR
YSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYCARLGRKPSGSVAFDIWWQGTMV
TVSS

Ab65 – Light chain variable region (SEQ ID NO:356)
EIVLTQSPATLSVSPGERATLSCRASQSVSYLAWYQQKPGQPRLSIIYDASNRTGIPAR
FSGSAGSFGTDFLTISLQSEDFAVYYCQDQDDYTFQGGTKEIK
Ab66 – Heavy chain variable region (SEQ ID NO:357)
QVQLVQSGAEVKPGASVKVSCKASGYTFTGSYMHVRQAPGQGLEWMGWIPNSG
GTNYAQQFQGRVTMTRDTSISTAYMELSRLRSDDTVAYYCARAGHKTHDYWGQGTLV
TVSS

Ab66 – Light chain variable region (SEQ ID NO:358)
EIVLTQSPGTLSLSPGERATLSCRASQSVSYLAWYQQQPGQAPRLLIYDANRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQRSAVPITFGGGTKVEIK

Ab67 – Heavy chain variable region (SEQ ID NO:326)
QVQLVQSGAEVKPGASVKVSCKAGYFTFSYMYMVHRQAPGQGLEWMGIINPSGGS
TTYAQQFQGRVTMTRDSTSTTVYMELSSLRSDDTAVYVACRGKSMDVWGQGTTTVT
SS

Ab67 – Light chain variable region (SEQ ID NO:359)
EIVLTQSPATLSLSPGERATLSCRASQSVSYLAWYQQQPGQAPRLLIYDANRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQRSHFPITFGGGTKVEIK

Ab68 – Heavy chain variable region (SEQ ID NO:360)
QVQLVESGGSVGVQPGRSLRSLCAASGFTSSYGMHWVRQAPGQGLEWVALIWDGSN
KYYADSVKGRFTISRDSKNTLYSLQMNSLRAEDTAVYVYACPKGSMTDYWGQGTLVTV
SS

Ab68 – Light chain variable region (SEQ ID NO:361)
EIVLTQSPATLSLSPGERATLSCRASQSVSYLAWYQQQPGQAPRLLIYDANRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRANYPITFGGGTKVEIK
Ab69 – Heavy chain variable region (SEQ ID NO:362)
QVQLVQSGAEVKPGASVKVSCKASGYTFTGSYMHWVRQAPGQGLEWMGWIPNSG
GTNYAQQFQGRVTMTDRTSTAYMELSRLSDDTAVYYCARAKSVDHDYWGGTVL
TVSS

Ab69 – Light chain variable region (SEQ ID NO:363)
EIVLTQSPATLSLSPGERATLSCRAQSVSSYLAWYQQKPGQQPRLLIYDASNRTGI
PARFSGSGGSFTFTLTISSLEPEDFAVYYCQQRADYPITFGGGTKVEIK

Ab70 – Heavy chain variable region (SEQ ID NO:345)
QVQLVQSGAEVKPGASVKVSCKASGYTFTGSYMHWVRQAPGQGLEWMGWIPNSG
GTSAQQKFQGRVTMTDRTSTAYMELSRLSDDTAVYYCARASKMGDDWGQGL
TVSS

Ab70 – Light chain variable region (SEQ ID NO:364)
EIVLTQSPATLSLSPGERATLSCRAQSVSSYLAWYQQKPGQQPRLLIYDASNRTGI
PARFSGSGGSFTFTLTISSLEPEDFAVYYCQQRADYPITFGGGTKVEIK

Ab71 – Heavy chain variable region (SEQ ID NO:365)
QVQLVQSGAEVKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIIPSGGS
TSYAQQKFQGRVTMTDRTSTSTVYMELSSLRSEDTAVYYCARDISTHDYDLAFDI
WGGT MTVSS

Ab71 – Light chain variable region (SEQ ID NO:366)
EIVMTQSPGTLTSLSPGERATLSCRAQSVSSSYLAWYQQKPGQQPRLLIYGASNRATGI
PADFSGSGSFTFTLTISSLEPEDFAVYYCQQAGSHPFITFGGGTKVEIK
Ab72 – Heavy chain variable region (SEQ ID NO:367)
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGSIYYSGSTNYNP
SLKSRVTISVTDTSKQFSLKLSSVTADAATAVAYYCARSGETELFDYWGQGTLVTVSS

Ab72 – Light chain variable region (SEQ ID NO:368)
DIQMTQPSSLSASVGDRVTITCQASQDITNYLNWYYQQPKGAPKLLIYDASNLETGVPS
RFGSRSQTDFDTFTISSLQPEDIATYQCQDYNYPFTFGGKTVEIK

Ab73 – Heavy chain variable region (SEQ ID NO:370)
EVQLVQGAEVKPGESLKLISCKGSYGTSFTYSYWGVRQMPGKGLEWMGIIYPGDSDTT
YSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYCARAKMLDDGYAFDIWQGTMV
TVSS

Ab73 – Light chain variable region (SEQ ID NO:370)
EIVMTQSPATLSVSPGERATLSERASQSVSSNLAWYQQKPGAPRLLIIYGASTATGIPA
RFSGSGSEFTLTISLQSEDFAVYCYCQDDNYPYTFGGGTKEIK

Ab74 – Heavy chain variable region (SEQ ID NO:357)
QVQLVQSGAEVKPGASVKSCKASGYTFTGSYMHWVRQAPGQGLEWMGWINPNSG
GTNYAQKFGQRVTMTRDTSISTAYMELSRLRSDDTAVYYCARAGHKTHDYWGQGTLV
TVSS

Ab74 – Light chain variable region (SEQ ID NO:371)
EIVMTQSPATLSVSPGERATLSERASQSVSSNLAWYQQKPGAPRLLIIYGASTATGIPA
RFSGSGSEFTLTISLQSEDFAVYCYCQDDNYPYTFGGGTKEIK

Ab75 – Heavy chain variable region (SEQ ID NO:372)
QVQLVQSGAEVKPGASVKSCKASGYTFTGYYMHVRQAPGQGLEWMGWINPNSG
GTNYAQKFGQRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDLGYSSLALDIWGQG
TMVTVSS
Ab75 – Light chain variable region (SEQ ID NO:373)
EIVLTQSPATLSLPGERATLSCRAQSQSVSYLLAWYQQKPGQAPRLLYDASNRATGIPAR
FSGSGGSGTDFLTIISLEPEDFAVYYCQQVSNYPFTFGGGTKVEIK

Ab76 – Heavy chain variable region (SEQ ID NO:374)
EVQLVESGGGLVKPGSLRLSCAASGFASYSMMNWVRQAPGKGLEWVSPSSISSHSSYIYY
ADSVKGRFTISRDNAKLNLQMMNLRADATAVYYCARGGGRRGDNNWFDPGQGTL
VTVSS

Ab76 – Light chain variable region (SEQ ID NO:375)
DIVMTQSPDSLAVSLGERATINCKSSQSVMYSSKNNKNLAWYQQKPGQPPKLIYWAST
RESGVPDRFSGSGSTDFLTISSLQAEDVAVYYCQQYHDAPITFGGGTKVEIK

Ab77 – Heavy chain variable region (SEQ ID NO:376)
QVQLVESGGGVVQPGRLRLCAASGFASYSMHWVRQAPGKGLEWVAVISYDGSNK
YYADSVKGRFTISRDNSKNTLQLMNLSLRAEDTAVYYCARGPPHEMDYWGQGTL
VTSS

Ab77 – Light chain variable region (SEQ ID NO:377)
DIVMTQSPDSLAVSLGERATINCKSSQSVMYSSKNNKNLAWYQQKPGQPPKLIYWAST
RESGVPDRFSGSGSTDFLTISSLQAEDVAVYYCQQAYVVPPTFGGGTKVEIK

Ab78 – Heavy chain variable region (SEQ ID NO:378)
QVQLVESGGGVVQPGRLRLCAASGFASYSMHWVRQAPGKGLEWVAVIWDGSNK
KYYADSVKGRFTISRDNSKNTLQLMNLSLRAEDTAVYYCARTPYPWIYFDLDWGRGTL
VTSS
Ab78 – Light chain variable region (SEQ ID NO:379)
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGLTFTFLTISSLEPEDFAVYYCQQADNWPFITFGGGTKVEIK

Ab79 – Heavy chain variable region (SEQ ID NO:380)
EVQLVESGGGLVQPGGSLRLSCAASGFTSSYSMNWVRQAPGKGLEWVSYISGSSSTYI
YADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARGGRRHYGGMDVWQGTTVTSS

Ab79 – Light chain variable region (SEQ ID NO:381)
DIVMTQSPSLPVTPEGAPASISCRSSQSLHNSGYNYLDWLYQLKPGQSPQLLILYLGSHRAS
GVPDRSFSGSGLTFTLKI NRFVEAEEDVGVYMYCMQALESRTFGGGGTKVEIK

Ab80 – Heavy chain variable region (SEQ ID NO:382)
QVQLVQSGAEVKPGSSVKVSCKASGGTFSSYAI SWVRQAPGQGLEWMMGGIIPIFGTAN
YAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARGGTFWSGSWALYWQGTL
VTSS

Ab80 – Light chain variable region (SEQ ID NO:383)
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGLTFTFLTISSLEPEDFAVYYCQQYVNWPFTFGGGTKVEIK

Ab81 – Heavy chain variable region (SEQ ID NO:384)
QVQLVQSGAEVKPGSSVKVSCKASGGTFSSYAI SWVRQAPGQGLEWMMGGIIPIFGTAN
YAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARDSGN YDYWS GALRYWQG
TLVTSS
Ab81 – Light chain variable region (SEQ ID NO:385)
EIVLTQSPATLSLPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRTAGIPAR
FSGSGSTDFLTLISSLEPEDFAVYCYQQSSNWPFWTFGGGTKEIK

Ab82 – Heavy chain variable region (SEQ ID NO:386)
QVQLQESGPGLVKPSQTLSLCTVSGGSISSLGGYYWSWIRQPHEGKLEWIGIYYSSGTV
YNPSLKSRTVTISVDTSDKNQFSKLSSLVTAADTAVYYCARVSSSWYKAWQGQTMVTVSS

Ab82 – Light chain variable region (SEQ ID NO:387)
DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPQGKAPKLLIYAASSLQSGVPS
RFSGSGSSTDFLTLISSLEPEDFATYYCQQASTFPITFGGGTKVEIK

Ab83 – Heavy chain variable region (SEQ ID NO:388)
QVQLQWAGLKLKPSETLSLCTAVYGGSFSGYYWSWIRQPHEGKLEWIGEIDHSSTKY
NPSLKSRTVTISVDTSDKNQFSKLSSLVTAADTAVYYCARVGVVGRPGYSADFJWQGT
MTVTSS

Ab83 – Light chain variable region (SEQ ID NO:389)
DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPQGKAPKLLIYAASSLQSGVPS
RFSGSGSSTDFLTLISSLEPEDFATYYCQQRNSLPLTFGGGTKVEIK

Ab84 – Heavy chain variable region (SEQ ID NO:351)
QVQLVQSGAEVKPGASVKVSCKASGYTFTSYGJSGWVRQPQGQLEWMGWISTYNGN
NTYAQKLLQGRVMTTDDTSSTAYMELRSLRSDDTAVYYCARSGSRYDSWYDQGT
LVTVSS

Ab84 – Light chain variable region (SEQ ID NO:390)
DIQMTQSPSSLSASVGDRVTTTTCRASQSISSYLNWYQQKPQGKAPKLLIYAASSLQSGVPSR
FSGSGSTDFLTLISSLEPEDFATYYCQQSYDFPITFGGGTKVEIK
Ab85 – Heavy chain variable region (SEQ ID NO:391)
QVQLVESGGGVVQPGSRSLRLSCAASGFITFSSYGMHWVRQAPGKGLEWVAVIWyDGSN
KYYADSKVGRFTISRDNSKNTLQLQMNSLAEDTAVYYCACKDLLGGYGYAALGMDDV
WGQGTTTVSS

Ab85 – Light chain variable region (SEQ ID NO:392)
DIQLTGSPSSVSASVGRVTITCRASQDISSWLAWYQQKPGKAPKLILYAAASSLQSGVPSRFSGSGSGTDTFTLTISLQPEDFATYYCQEQYVDYPLTFGGGKVEIK

Ab86 – Heavy chain variable region (SEQ ID NO:393)
QVQLVESGGGVVQPGSRSLRLSCAASGFITFSSYGMHWVRQAPGKGLEWVAVISYDGSNK
YYADSVKGRFTISRDNSKNTLQLQMNSLAEDTAVYYCAKDGVYVYGLGNWFDPWGQG
TLVTTVSS

Ab86 – Light chain variable region (SEQ ID NO:394)
DIQMTQSPSTLSASVGRVTITCRASQSISSWLAWYQQKPGKAPKLILYKAJESSLSGVPSRFSGSGSGTEFTLTISLQPDDDFATYYCQLNSYSPFTGGGKVEIK

Ab87 – Heavy chain variable region (SEQ ID NO:395)
QVQLQESGPGLVKPSETLSLTCTVSGSISISYYWSWIRQPPPQKGLGWIGISYYSIGTNYNP
SLKSRVTISVDTSDKNFSLKLSSVTAADTAVYHCARRHGWDVGFDPWGGQGTLVTVSS

Ab87 – Light chain variable region (SEQ ID NO:396)
EIILTQSPATLSLSPGERATLSRCSRASQSVRSLAWYQQKPGQAPRLLYIDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQYIFWPPTFGGKVEIK
CLAIMS

What is claimed is:

1. An isolated agonist antibody that binds to a TREM2 protein, wherein the antibody induces one or more TREM2 activities.

2. The isolated antibody of claim 1, wherein the TREM2 protein is a mammalian protein or a human protein.

3. The isolated antibody of claim 2, wherein the TREM2 protein is a wild-type protein.

4. The isolated antibody of claim 2, wherein the TREM2 protein is a naturally occurring variant.

5. The isolated antibody of any one of claims 1-4, wherein the TREM2 protein is expressed on human dendritic cells, human macrophages, human monocytes, human osteoclasts, human Langerhans cells of skin, human Kupffer cells, and/or human microglia.

6. The isolated antibody of any one of claims 1-5, wherein the isolated antibody induces or retains TREM2 clustering on a cell surface.

7. The isolated antibody of any one of claims 1-6, wherein the one or more TREM2 activities comprise TREM2 binding to DAP12.

8. The isolated antibody of any one of claims 1-7, wherein the one or more TREM2 activities comprise TREM2 autophosphorylation.

9. The isolated antibody of any one of claims 1-8, wherein the one or more TREM2 activities comprise DAP12 phosphorylation.

10. The isolated antibody of claim 8 or claim 9, wherein the TREM2 autophosphorylation, the DAP12 phosphorylation, or both is induced by one or more SRC family tyrosine kinases.

11. The isolated antibody of claim 10, wherein the one or more SRC family tyrosine kinases comprise a Syk kinase.

12. The isolated antibody of any one of claims 1-11, wherein the one or more TREM2 activities comprise PI3K activation.
13. The isolated antibody of any one of claims 1-12, wherein the one or more TREM2 activities comprise increased expression of one or more anti-inflammatory cytokines.

14. The isolated antibody of any one of claims 1-12, wherein the one or more TREM2 activities comprise increased expression of one or more cytokines selected from the group consisting of IL-12p70, IL-6, and IL-10.

15. The isolated antibody of claim 13 or claim 14, wherein the increased expression occurs in one or more cells selected from the group consisting of macrophages, dendritic cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and microglial cells.

16. The isolated antibody of any one of claims 1-15, wherein the one or more TREM2 activities comprise reduced expression of one or more pro-inflammatory cytokines.

17. The isolated antibody of any one of claims 1-15, wherein the one or more TREM2 activities comprise reduced expression of one or more pro-inflammatory mediators selected from the group consisting of IFN-α, IFN-β, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, and CRP.

18. The isolated antibody of any one of claims 1-16, wherein the one or more TREM2 activities comprise reduced expression of TNF-α, IL-6, or both.

19. The isolated antibody of claim 16 or claim 18, wherein the reduced expression of the one or more pro-inflammatory mediators occurs in one or more cells selected from the group consisting of macrophages, dendritic cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and microglial cells.

20. The isolated antibody of any one of claims 1-18, wherein the one or more TREM2 activities comprise extracellular signal-regulated kinase (ERK) phosphorylation.

21. The isolated antibody of any one of claims 1-20, wherein the one or more TREM2 activities comprise increased expression of C-C chemokine receptor 7 (CCR7).
22. The isolated antibody of any one of claims 1-21, wherein the one or more TREM2 activities comprise induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells.

23. The isolated antibody of any one of claims 1-22, wherein the one or more TREM2 activities comprise an increased ability of dendritic cells, monocytes, microglia, and/or macrophages to induce T-cell proliferation.

24. The isolated antibody of any one of claims 1-22, wherein the one or more TREM2 activities comprise an enhancement, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation.

25. The isolated antibody of any one of claims 1-24, wherein the one or more TREM2 activities comprise induction of osteoclast production, increased rate of osteoclastogenesis, or both.

26. The isolated antibody of any one of claims 1-25, wherein the one or more TREM2 activities comprise increasing the survival of dendritic cells, macrophages, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or microglia.

27. The isolated antibody of any one of claims 1-26, wherein the one or more TREM2 activities comprise increasing the function of dendritic cells, macrophages, and/or microglia.

28. The isolated antibody of any one of claims 1-27, wherein the one or more TREM2 activities comprise increasing phagocytosis by dendritic cells, macrophages, monocytes, and/or microglia under conditions of reduced levels of M-CSF.

29. The isolated antibody of any one of claims 1-28, wherein the one or more TREM2 activities comprise decreasing phagocytosis by dendritic cells, macrophages, monocytes, and/or microglia in the presence of normal levels of M-CSF.

30. The isolated antibody of any one of claims 26-29, wherein the macrophages and/or microglia are M1 macrophages and/or microglia, M2 macrophages and/or microglia, or both.

31. The isolated antibody of claim 30, wherein the M1 macrophages and/or microglia are activated M1 macrophages and/or microglia.
32. The isolated antibody of any one of any one of claims 1-31, wherein the one or more TREM2 activities comprise induction of one or more types of clearance selected from the group consisting of apoptotic neuron clearance, nerve tissue debris clearance, non-nerve tissue debris clearance, bacteria or other foreign body clearance, disease-causing protein clearance, and tumor cell clearance.

33. The isolated antibody of any one of claims 1-32, wherein the one or more TREM2 activities comprise induction of phagocytosis of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, disease-causing proteins, disease-causing peptides, disease-causing nucleic acids, or tumor cells.

34. The isolated antibody of claim 33, wherein the disease-causing nucleic acid is antisense GGCCCC (G2C4) repeat-expansion RNA.

35. The isolated antibody of claim 32 or claim 33, wherein the disease-causing protein is selected from the group consisting of amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein A1, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, and proline-arginine (PR) repeat peptides.

36. The isolated antibody of any one of claims 1-35, wherein the one or more TREM2 activities comprise normalization of disrupted TREM2/DAP12-dependent gene expression.

37. The isolated antibody of any one of claims 1-36, wherein the one or more TREM2 activities comprise recruitment of Syk, ZAP70, or both to a DAP12/TREM2 complex.

38. The isolated antibody of any one of claims 1-37, wherein the one or more TREM2 activities comprise Syk phosphorylation.
39. The isolated antibody of any one of claims 1-38, wherein the one or more TREM2 activities comprise increased expression of CD83 and/or CD86 on dendritic cells, monocytes, macrophages, or both.

40. The isolated antibody of claim 39, wherein the dendritic cells are bone marrow-derived dendritic cells.

41. The isolated antibody of any one of claims 1-40, wherein the one or more TREM2 activities comprise reduced secretion of one or more inflammatory cytokines.

42. The isolated antibody of 41, wherein the one or more inflammatory cytokines are selected from the group consisting of IFN-α, IFN-β, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, CRP, and MCP-1.

43. The isolated antibody of any one of claims 1-41, wherein the one or more TREM2 activities comprise reduced expression of one or more inflammatory receptors.

44. The isolated antibody of claim 43, wherein the one or more inflammatory receptors comprise CD86.

45. The isolated antibody of any one of claims 1-29, wherein the one or more TREM2 activities comprise increasing activity of one or more TREM2-dependent genes.

46. The isolated antibody of claim 45, wherein the one or more TREM2-dependent genes comprise one or more nuclear factor of activated T-cells (NFAT) transcription factors.

47. The isolated antibody of any one of claims 1-29, wherein the antibody is of the IgG class the IgM class, or the IgA class.

48. The isolated antibody of claim 47, wherein the antibody is of the IgG class and has an IgG1, IgG2, IgG3, or IgG4 isotype.

49. The isolated antibody of claim 47, wherein the antibody has an IgG2 isotype.

50. The isolated antibody of claim 49, wherein the antibody comprises a human IgG2 constant region.
51. The isolated antibody of claim 50, wherein the human IgG2 constant region comprises an Fc region.

52. The isolated antibody of any one of claims 49-51, wherein the antibody induces the one or more TREM2 activities independently of binding to an Fc receptor.

53. The isolated antibody of any one of claims 49-51, wherein the antibody binds an inhibitory Fc receptor.

54. The isolated antibody of claim 53, wherein the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγRII).

55. The isolated antibody of claim 53 or claim 54, wherein the human IgG2 constant region comprises an Fc region that comprises one or more modifications.

56. The isolated antibody of claim 55, wherein the Fc region comprises one or more amino acid substitutions.

57. The isolated antibody of claim 56, wherein the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of V234A, G237A, H268Q, V309L, A330S, P331S, C232S, C233S, S267E, L328F, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

58. The isolated antibody of claim 50, wherein the human IgG2 constant region comprises a light chain constant region comprising a C214S amino acid substitution, wherein the numbering of the residues is according to EU numbering.

59. The isolated antibody of claim 47, wherein the antibody has an IgG1 isotype.

60. The isolated antibody of claim 59, wherein the antibody comprises a human IgG1 constant region.

61. The isolated antibody of claim 60, wherein the human IgG1 constant region comprises an Fc region.

62. The isolated antibody of any one of claims 59-61, wherein the antibody binds an inhibitory Fc receptor.
63. The isolated antibody of claim 62, wherein the inhibitory Fc receptor is inhibitory Fc-
gamma receptor IIB (FcγRIIB).

64. The isolated antibody of any one of claims 61-63, wherein the Fc region comprises one or
more modifications.

65. The isolated antibody of claim 64, wherein the Fc region comprises one or more amino
acid substitutions.

66. The isolated antibody of claim 65, wherein the one or more amino acid substitutions in
the Fc region are at a residue position selected from the group consisting of N297A, D265A,
A330L, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the
residues is according to EU numbering.

67. The isolated antibody of any one of claims 61-63, wherein the antibody comprises an
IgG2 isotype heavy chain constant domain 1(\text{CH1}) and hinge region.

68. The isolated antibody of claim 67, wherein the IgG2 isotype CH1 and hinge region
comprise the amino acid sequence of ASTKGPSVFP LAPCSRSTSE STAALGCLVK
DYFPEPVTVS WNSGALTSGVHTFPAVLQSS GLYSLSSVVT VPSSNFQTQT
YTCNVDHKPS NTKVDKTVERKCCVECPPCP (SEQ ID NO: 397).

69. The isolated antibody of claim 67 or claim 68, wherein the antibody Fc region comprises
a S267E amino acid substitution, a L328F amino acid substitution, or both, and/or a N297A or
N297Q amino acid substitution, wherein the numbering of the residues is according to EU
numbering.

70. The isolated antibody of claim 59, wherein the antibody comprises a mouse IgG1
constant region.

71. The isolated antibody of claim 47, wherein the antibody has an IgG4 isotype.

72. The isolated antibody of claim 71, wherein the antibody comprises a human IgG4
constant region.
73. The isolated antibody of claim 72, wherein the human IgG4 constant region comprises an Fc region.

74. The isolated antibody of any one of claims 71-73, wherein the antibody binds an inhibitory Fc receptor.

75. The isolated antibody of claim 74, wherein the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcyRIIB).

76. The isolated antibody of any one of claims 73-75, wherein the Fc region comprises one or more modifications.

77. The isolated antibody of claim 76, wherein the Fc region comprises one or more amino acid substitutions.

78. The isolated antibody of claim 77, wherein the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of L235A, G237A, S228P, L236E, S267E, E318A, L328F, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

79. The isolated antibody of claim 47, wherein the antibody has a hybrid IgG2/4 isotype.

80. The isolated antibody of claim 79, wherein the antibody comprises an amino acid sequence comprising amino acids 118 to 260 of human IgG2 and amino acids 261 to 447 of human IgG4, wherein the numbering of the residues is according to EU numbering.

81. The isolated antibody of claim 71, wherein the antibody comprises a mouse IgG4 constant region.

82. The isolated antibody of any one of claims 1-81, wherein the isolated antibody is an antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of human TREM2, and wherein the antibody fragment is cross-linked to a second antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of human TREM2.
83. The isolated antibody of claim 82, wherein the fragment is an Fab, Fab’, Fab’-SH, F(ab’)2, Fv or scFv fragment.

84. An isolated inert antibody that binds to a TREM2 protein.

85. An isolated antagonist antibody that binds to a TREM2 protein.

86. The isolated antibody of claim 84 or claim 85, wherein the TREM2 protein is a mammalian protein or a human protein.

87. The isolated antibody of claim 86, wherein the TREM2 protein is a wild-type protein.

88. The isolated antibody of claim 86, wherein the TREM2 protein is a naturally occurring variant.

89. The isolated antibody of claim 86, wherein the TREM2 protein is a disease variant.

90. The isolated antibody of any one of claims 85-89, wherein the isolated antibody inhibits one or more TREM2 activities.

91. The isolated antibody of claim 90, wherein the one or more TREM2 activities comprise decreasing activity of one or more TREM2-dependent genes.

92. The isolated antibody of claim 91, wherein the one or more TREM2-dependent genes comprise one or more nuclear factor of activated T-cells (NFAT) transcription factors.

93. The isolated antibody of any one of claims 90-92, wherein the one or more TREM2 activities comprise decreasing the survival of macrophages, microglial cells, M1 macrophages, M1 microglial cells, M2 macrophages, M2 microglial cells, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or dendritic cells.

94. The isolated antibody of any one of claims 85-93, wherein the isolated antibody inhibits interaction between TREM2 and one or more TREM2 ligands, inhibits TREM2 signal transduction, or both.

95. The isolated antibody of any one of claims 84-94, wherein the antibody is incapable of binding an Fc-gamma receptor (FcyR).

96. The isolated antibody of claim 95, wherein the antibody has an IgG1 isotype.
97. The isolated antibody of claim 96, wherein the antibody comprises a human IgG1 constant region.

98. The isolated antibody of claim 97, wherein the human IgG1 constant region comprises an Fc region.

99. The isolated antibody of claim 98, wherein the Fc region comprises one or more modifications.

100. The isolated antibody of claim 99, wherein the Fc region comprises one or more amino acid substitutions.


102. The isolated antibody of claim 101, wherein the Fc region further comprises an amino acid deletion at a position corresponding to glycine 236 according to EU numbering.

103. The isolated antibody of claim 96, wherein the antibody comprises a mouse IgG1 constant region.

104. The isolated antibody of claim 95, wherein the antibody has an IgG2 isotype.

105. The isolated antibody of claim 104, wherein the antibody comprises a human IgG2 constant region.

106. The isolated antibody of claim 105, wherein the human IgG2 constant region comprises an Fc region.

107. The isolated antibody of claim 106, wherein the Fc region comprises one or more modifications.

108. The isolated antibody of claim 107, wherein the Fc region comprises one or more amino acid substitutions.
109. The isolated antibody of claim 108, wherein the one or more amino acid substitutions in
the Fc region are at a residue position selected from the group consisting of V234A, G237A,
any combination thereof, wherein the numbering of the residues is according to EU numbering.

110. The isolated antibody of claim 95 wherein the antibody has an IgG4 isotype.

111. The isolated antibody of claim 110, wherein the antibody comprises a human IgG4
constant region.

112. The isolated antibody of claim 111, wherein the human IgG4 constant region comprises
an Fc region.

113. The isolated antibody of claim 112, wherein the Fc region comprises one or more
modifications.

114. The isolated antibody of claim 113, wherein the Fc region comprises one or more amino
acid substitutions.

115. The isolated antibody of claim 114, wherein the one or more amino acid substitutions in
the Fc region are at a residue position selected from the group consisting of E233P, F234V,
N297A, N297Q, and any combination thereof, wherein the numbering of the residues is
according to EU numbering.

116. The isolated antibody of any one of claims 84-115, wherein the isolated antibody is an
antibody fragment that binds to one or more human proteins selected from the group consisting
of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of
TREM2.

117. The isolated antibody of claim 116, wherein the fragment is an Fab, Fab’, Fab’-SH,
F(ab’)2, Fv or scFv fragment.

118. The isolated antibody of claim 66, claim 101, or claim 102, wherein the Fc region further
comprises one or more additional amino acid substitutions at a position selected from the group
consisting of A330L, L234F; L235E, P331S, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

119. The isolated antibody of any one of claims 55-118, wherein the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

120. The isolated antibody of claim 78 or claim 115, wherein the Fc region further comprises a S228P amino acid substitution according to EU numbering.

121. The isolated antibody of any one of claims 1-120, wherein the antibody competes for binding of TREM2 with one or more TREM2 ligands.

122. The isolated antibody of claim 121, wherein the one or more TREM2 ligands are selected from the group consisting of E. coli cells, apoptotic cells, nucleic acids, anionic lipids, zwitterionic lipids, negatively charged phospholipids, phosphatidylserine, sulfatides, phosphatidylycholin, sphingomyelin, membrane phospholipids, lipidated proteins, proteolipids, lipidated peptides, and lipidated amyloid beta peptide.

123. The isolated antibody of any one of claims 1-120, wherein the antibody does not compete for binding of TREM2 with a TREM2 ligand.

124. The isolated antibody of any one of claims 1-123, wherein the antibody is a human antibody, a humanized antibody, a bispecific antibody, a multivalent antibody, a conjugated antibody, or a chimeric antibody.

125. The isolated antibody of any one of claims 1-124, wherein the antibody is a bispecific antibody recognizing a first antigen and a second antigen.

126. The isolated antibody of claim 125, wherein the first antigen is human TREM2 or a naturally occurring variant thereof, and the second antigen is a disease-causing protein selected from the group consisting of: amyloid beta or fragments thereof, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein A1, serum
amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, and proline-arginine (PR) repeat peptides; a blood brain barrier targeting protein selected from the group consisting of: transferrin receptor, insulin receptor, insulin like growth factor receptor, LRP-1, and LRP1; or ligands and/or proteins expressed on immune cells, wherein the ligands and/or proteins selected from the group consisting of: CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, BTLA, KIR, GAL9, TIM3, A2AR, LAG, and phosphatidylinerse.

127. The isolated antibody of claim 125, wherein the first antigen is human TREM2 or a naturally occurring variant thereof, and the second antigen is a protein expressed on one or more tumor cells.

128. The isolated antibody of any one of claims 1-122, wherein the antibody is an antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, human DAP12, and naturally occurring variant of human DAP12; and wherein the antibody is used in combination with one or more antibodies that specifically bind a disease-causing protein selected from the group consisting of: amyloid beta, Tau, IAPP, alpha-synuclein, TDP-43, FUS protein, prion protein, PrPSc, huntingtin, calcitonin, superoxide dismutase, ataxin, Lewy body, atrial natriuretic factor, islet amyloid polypeptide, insulin, apolipoprotein AI, serum amyloid A, medin, prolactin, transthyretin, lysozyme, beta 2 microglobulin, gelsolin, keratoepithelin, cystatin, immunoglobulin light chain AL, S-IBM protein, Repeat-associated non-ATG (RAN) translation products, DiPeptide repeat (DPR) peptides, glycine-alanine (GA) repeat peptides, glycine-proline (GP) repeat peptides, glycine-arginine (GR) repeat peptides, proline-alanine (PA) repeat peptides, and proline-arginine (PR) repeat peptides, and any combination thereof, or with one or more antibodies that specifically bind a cancer-associated protein selected from the group consisting of: CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, GITR, PD-L1, CTLA4, PD-L2, PD-1,
B7-H3, B7-H4, HVEM, BTLA, KIR, GAL9, TIM3, A2AR, LAG, phosphatidylserine, and any combination thereof.

129. The isolated antibody of any one of claims 1-125, wherein the antibody is a monoclonal antibody.

130. The isolated antibody of any one of claims 1-129, wherein the antibody binds to one or more amino acids within amino acid residues selected from the group consisting of:
   i. amino acid residues 130-171 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 130-171 of SEQ ID NO: 1;
   ii. amino acid residues 139-153 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 139-153 of SEQ ID NO: 1;
   iii. amino acid residues 139-146 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 139-146 of SEQ ID NO: 1;
   iv. amino acid residues 130-144 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 130-144 of SEQ ID NO: 1;
   and
   v. amino acid residues 158-171 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 158-171 of SEQ ID NO: 1.

131. The isolated antibody of any one of claims 1-129, wherein the antibody binds to an epitope comprising one or more amino acids within amino acid residues selected from the group consisting of:
   i. amino acid residues 130-171 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 130-171 of SEQ ID NO: 1;
   ii. amino acid residues 139-153 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 139-153 of SEQ ID NO: 1;
   iii. amino acid residues 139-146 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 139-146 of SEQ ID NO: 1;
iv. amino acid residues 130-144 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 130-144 of SEQ ID NO: 1; and

v. amino acid residues 158-171 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 158-171 of SEQ ID NO: 1.

132. The isolated antibody of claim 130 or claim 131, wherein the antibody binds to an epitope further comprising one or more amino acid residues selected from the group consisting of:

i. amino acid residue Arg47 or Asp87 of SEQ ID NO: 1;

ii. amino acid residues 40-44 of SEQ ID NO: 1;

iii. amino acid residues 67-76 of SEQ ID NO: 1; and

iv. amino acid residues 114-118 of SEQ ID NO: 1.

133. The isolated antibody of any one of claims 1-131, wherein the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an HVR-H1, HVR-H2, and/or HVR-H3 of a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87, and/or wherein the light chain variable domain comprises an HVR-L1, HVR-L2, and/or HVR-L3 of a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87.
134. The isolated antibody of claim 133, wherein the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24.

135. The isolated antibody of claim 133 or claim 134, wherein the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49.

136. The isolated antibody of any one of claims 133-135, wherein the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119.

137. The isolated antibody of any one of claims 133-136, wherein the HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137.

138. The isolated antibody of any one of claims 133-137, wherein the HVR-L2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152.

139. The isolated antibody of any one of claims 133-138, wherein the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236.

140. The isolated antibody of any one of claims 1-131, wherein the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises:

   (a) an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24;

   (b) an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 25-49; and

   (c) an HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 50-119; and/or

wherein the light chain variable domain comprises:

   (a) an HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137;
(b) an HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152; and
(c) an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236 or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152.

141. An isolated anti-human TREM2 antibody, wherein the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and/or HVR-H3 of a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87, and/or wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and/or HVR-L3 of a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87.

142. The isolated antibody of claim 141, wherein the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24.

143. The isolated antibody of claim 141 or claim 142, wherein the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49.
144. The isolated antibody of any one of claims 141-143, wherein the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119.

145. The isolated antibody of any one of claims 141-144, wherein the HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137.

146. The isolated antibody of any one of claims 141-145, wherein the HVR-L2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152.

147. The isolated antibody of any one of claims 141-146, wherein the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236.

148. The isolated antibody of claim 141, wherein the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24, an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49, and an HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119, and/or wherein the light chain variable domain comprises an HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137, an HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152, and an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236.

149. An isolated anti-human TREM2 antibody which binds essentially the same TREM2 epitope as a monoclonal antibody selected from the group consisting of: Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, Ab36, Ab37, Ab38, Ab39, Ab40, Ab41, Ab42, Ab43, Ab44, Ab45, Ab46, Ab47, Ab48, Ab49, Ab50, Ab51, Ab53, Ab54, Ab55, Ab56, Ab57, Ab58, Ab59, Ab60, Ab61, Ab62, Ab63, Ab64, Ab65, Ab66, Ab67, Ab68, Ab69, Ab70, Ab71, Ab72, Ab73, Ab74, Ab75, Ab76, Ab77, Ab78, Ab79, Ab80, Ab81, Ab82, Ab83, Ab84, Ab85, Ab86, and Ab87.

sf-3499172 -272-
150. An isolated anti-human TREM2 antibody, wherein the isolated antibody comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises:

(a) an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:3-24;
(b) an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:25-49, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 25-49; and
(c) an HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:50-119, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 50-119; and/or

wherein the light chain variable domain comprises:

(a) an HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs:120-137;
(b) an HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:138-152, or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152; and
(c) an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153-236 or an amino acid sequence with at least about 95% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-152.

151. The isolated antibody of any one of claims 141-150, wherein the antibody is an agonist antibody, and wherein the antibody induces one or more TREM2 activities.

152. The isolated antibody of claim 151, wherein the isolated antibody induces or retains TREM2 clustering on a cell surface.

153. The isolated antibody of claim 151 or claim 152, wherein the one or more TREM2 activities are selected from the group consisting of TREM2 binding to DAP12; DAP12 binding
to TREM2; TREM2 phosphorylation, DAP12 phosphorylation; PI3K activation; increased expression of one or more cytokines selected from the group consisting of IL-12p70, IL-6, and IL-10; reduced expression of one or more pro-inflammatory mediators selected from the group consisting of IFN-a4, IFN-b, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, and CRP; reduced expression of TNF-α, IL-6, or both; extracellular signal-regulated kinase (ERK) phosphorylation; increased expression of C-C chemokine receptor 7 (CCR7); induction of microglial cell chemotaxis toward CCL19 and CCL21 expressing cells; increased ability of dendritic cells, monocytes, microglia, and/or macrophages to induce T-cell proliferation: an increase, normalization, or both of the ability of bone marrow-derived dendritic cells to induce antigen-specific T-cell proliferation; induction of osteoclast production, increased rate of osteoclastogenesis, or both; increasing the survival and/or function of one or more of dendritic cells, macrophages, M1 macrophages, activated M1 macrophages M2 macrophages, osteoclasts, Langerhans cells of skin, Kupffer cells, microglial cells, M1 microglial cells, activated M1 microglial cells, and M2 microglial cells; induction of one or more types of clearance selected from the group consisting of cancer cells clearance, apoptotic neuron clearance, nerve tissue debris clearance, non-nerve tissue debris clearance, bacteria or other foreign body clearance, and disease-causing protein clearance; induction of phagocytosis of one or more of apoptotic neurons, nerve tissue debris, non-nerve tissue debris, bacteria, other foreign bodies, disease-causing proteins or tumor cells; normalization of disrupted TREM2/DAP12-dependent gene expression; recruitment of Syk, ZAP70, or both to the TREM2/DAP12 complex; Syk phosphorylation; increased expression of CD83 and/or CD86 on dendritic cells, microglia, monocytes, or macrophages; reduced secretion of one or more inflammatory cytokines selected from the group consisting of IFN-a4, IFN-b, IL-1β, TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL-20 family members, IL-33, LIF, IFN-gamma, OSM, CNTF, TGF-beta, GM-CSF, IL-11, IL-12, IL-17, IL-18, CRP, and MCP-1; reduced expression of one or more inflammatory receptors; increasing phagocytosis by macrophages, monocytes, dendritic cells, and/or microglia under conditions of reduced levels of MCSF; decreasing phagocytosis by macrophages, monocytes, dendritic cells,
and/or microglia in the presence of normal levels of MCSF; increasing activity of one or more TREM2-dependent genes; and any combination thereof.

154. The isolated antibody of any one of claims 151-153, wherein the antibody is of the IgG class the IgM class, or the IgA class.

155. The isolated antibody of claim 154, wherein the antibody is of the IgG class and has an IgG1, IgG2, IgG3, or IgG4 isotype.

156. The isolated antibody of claim 155, wherein the antibody has an IgG2 isotype.

157. The isolated antibody of claim 156, wherein the antibody comprises a human IgG2 constant region.

158. The isolated antibody of claim 157, wherein the human IgG2 constant region comprises an Fc region.

159. The isolated antibody of any one of claims 156-158, wherein the antibody induces the one or more TREM2 activities independently of binding to an Fc receptor.

160. The isolated antibody of any one of claims 156-158, wherein the antibody binds an inhibitory Fc receptor.

161. The isolated antibody of claim 160, wherein the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcγIIIB).

162. The isolated antibody of claim 160 or claim 161, wherein the Fc region comprises one or more modifications.

163. The isolated antibody of claim 162, wherein the Fc region comprises one or more amino acid substitutions.

164. The isolated antibody of claim 163, wherein the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of V234A, G237A, H268Q, V309L, A330S, P331S, C232S, C233S, S267E, L328F, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering.
165. The isolated antibody of claim 157, wherein the human IgG2 constant region comprises a light chain constant region comprising a C214S amino acid substitution, wherein the numbering of the residues is according to EU numbering.

166. The isolated antibody of claim 155, wherein the antibody has an IgG1 isotype.

167. The isolated antibody of claim 166, wherein the antibody comprises a human IgG1 constant region.

168. The isolated antibody of claim 167, wherein the human IgG1 constant region comprises an Fc region.

169. The isolated antibody of any one of claims 166-168, wherein the antibody binds an inhibitory Fc receptor.

170. The isolated antibody of claim 169, wherein the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIB (FcyRIIB).

171. The isolated antibody of any one of claims 168-170, wherein the Fc region comprises one or more modifications.

172. The isolated antibody of claim 171, wherein the Fc region comprises one or more amino acid substitutions.

173. The isolated antibody of claim 172, wherein the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of N297A, D265A, L234A, L235A, G237A, C226S, C229S, E233P, L234V, L234F, L235E, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

174. The isolated antibody of any one of claims 168-170, wherein the antibody comprises an IgG2 isotype heavy chain constant domain 1(CH1) and hinge region.

175. The isolated antibody of claim 174, wherein the IgG2 isotype CH1 and hinge region comprise the amino acid sequence of ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYPFEPVTVS WNSGALTSGVHTFPAPVLQSS GLYSLSSVVT VPSSNGTQT YTCNVDHKPS NTKVDKTVKCCVECPPC (SEQ ID NO:397).
176. The isolated antibody of claim 174 or claim 175, wherein the antibody Fc region comprises a S267E amino acid substitution, a L328F amino acid substitution, or both, and/or a N297A or N297Q amino acid substitution, wherein the numbering of the residues is according to EU numbering.

177. The isolated antibody of claim 166, wherein the antibody comprises a mouse IgG1 constant region.

178. The isolated antibody of claim 155, wherein the antibody has an IgG4 isotype.

179. The isolated antibody of claim 178, wherein the antibody comprises a human IgG4 constant region.

180. The isolated antibody of claim 179, wherein the human IgG4 constant region comprises an Fc region.

181. The isolated antibody of any one of claims 178-180, wherein the antibody binds an inhibitory Fc receptor.

182. The isolated antibody of claim 181, wherein the inhibitory Fc receptor is inhibitory Fc-gamma receptor IIb (FcγIIb).

183. The isolated antibody of any one of claims 180-182, wherein the Fc region comprises one or more modifications.

184. The isolated antibody of claim 183, wherein the Fc region comprises one or more amino acid substitutions.

185. The isolated antibody of claim 184, wherein the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of L235A, G237A, S228P, L236E, S267E, E318A, L328F, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

186. The isolated antibody of claim 154, wherein the antibody has a hybrid IgG2/4 isotype.
187. The isolated antibody of claim 186, wherein the antibody comprises an amino acid sequence comprising amino acids 118 to 260 of human IgG2 and amino acids 261 to 447 of human IgG4, wherein the numbering of the residues is according to EU numbering.

188. The isolated antibody of claim 179, wherein the antibody comprises a mouse IgG4 constant region.

189. The isolated antibody of any one of claims 149-188, wherein the isolated antibody is an antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of TREM2, and wherein the antibody fragment is cross-linked to a second antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of TREM2.

190. The isolated antibody of claim 189, wherein the fragment is an Fab, Fab’, Fab’-SH, F(ab’)2, Fv or scFv fragment.

191. The isolated antibody of any one of claims 141-150, wherein the isolated antibody is an inert antibody.

192. The isolated antibody of any one of claims 141-150, wherein the isolated antibody is an antagonist antibody.

193. The isolated antibody of claim 192, wherein the isolated antibody inhibits one or more TREM2 activities.

194. The isolated antibody of claim 193, wherein the one or more TREM2 activities are selected from the group consisting of decreasing activity of one or more TREM2-dependent genes; decreasing activity of one or more nuclear factor of activated T-cells (NFAT) transcription factors; decreasing the survival of macrophages, microglial cells, monocytes, osteoclasts, Langerhans cells of skin, Kupffer cells, and/or dendritic cells; and any combination thereof.

195. The isolated antibody of any one of claims 192-194, wherein the isolated antibody inhibits interaction between TREM2 and one or more TREM2 ligands, inhibits TREM2 signal transduction, or both.
196. The isolated antibody of any one of claims 191-195, wherein the antibody is incapable of binding an Fc-gamma receptor (FcγR).

197. The isolated antibody of claim 196, wherein the antibody has an IgG1 isotype.

198. The isolated antibody of claim 197, wherein the antibody comprises a human IgG1 constant region.

199. The isolated antibody of claim 198, wherein the human IgG1 constant region comprises an Fc region.

200. The isolated antibody of claim 199, wherein the Fc region comprises one or more modifications.

201. The isolated antibody of claim 200, wherein the Fc region comprises one or more amino acid substitutions.


203. The isolated antibody of claim 202, wherein the Fc region further comprises an amino acid deletion at a position corresponding to glycine 236 according to EU numbering.

204. The isolated antibody of claim 197, wherein the antibody comprises a mouse IgG1 constant region.

205. The isolated antibody of claim 196, wherein the antibody has an IgG2 isotype.

206. The isolated antibody of claim 205, wherein the antibody comprises a human IgG2 constant region.

207. The isolated antibody of claim 206, wherein the human IgG2 constant region comprises an Fc region.
208. The isolated antibody of claim 207, wherein the Fc region comprises one or more modifications.

209. The isolated antibody of claim 208, wherein the Fc region comprises one or more amino acid substitutions.

210. The isolated antibody of claim 209, wherein the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of V234A, G237A, H268E, V309L, N297A, N297Q, A330S, P331S, C232S, C233S, M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

211. The isolated antibody of claim 196, wherein the antibody has an IgG4 isotype.

212. The isolated antibody of claim 211, wherein the antibody comprises a human IgG4 constant region.

213. The isolated antibody of claim 212, wherein the human IgG4 constant region comprises an Fc region.

214. The isolated antibody of claim 213, wherein the Fc region comprises one or more modifications.

215. The isolated antibody of claim 214, wherein the Fc region comprises one or more amino acid substitutions.

216. The isolated antibody of claim 215, wherein the one or more amino acid substitutions in the Fc region are at a residue position selected from the group consisting of E233P, F234V, L235A, G237A, E318A, S228P, L236E, S241P, L248E, T394D, M252Y, S254T, T256E, N297A, N297Q, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

217. The isolated antibody of any one of claims 141-216, wherein the isolated antibody is an antibody fragment that binds to one or more human proteins selected from the group consisting of human TREM2, a naturally occurring variant of human TREM2, and a disease variant of TREM2.
218. The isolated antibody of claim 217, wherein the fragment is an Fab, Fab’, Fab’-SH, F(ab’)2, Fv or scFv fragment.

219. The isolated antibody of claim 173, claim 202, or claim 203, wherein the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of A330L, L234F; L235E, P331S, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

220. The isolated antibody of any one of claims 163-219, wherein the Fc region further comprises one or more additional amino acid substitutions at a position selected from the group consisting of M252Y, S254T, T256E, and any combination thereof, wherein the numbering of the residues is according to EU numbering.

221. The isolated antibody of claim 185 or claim 216, wherein the Fc region further comprises a serine to proline amino acid substitution at position 228 according to EU numbering.

222. The isolated antibody of any one of claims 141-221, wherein the antibody is a human antibody, a humanized antibody, a bispecific antibody, a multivalent antibody, or a chimeric antibody.

223. The isolated antibody of any one of claims 141-222, wherein the antibody is a bispecific antibody recognizing a first antigen and a second antigen.

224. The isolated antibody of any one of claims 141-223, wherein the antibody is a monoclonal antibody.

225. The isolated antibody of any one of the preceding claims, wherein the isolated antibody binds specifically to both human TREM2 and mouse TREM2.

226. The isolated antibody of any one of the preceding claims, wherein the isolated antibody has dissociation constant (K_D) for human TREM2 and mouse TREM2 that ranges from less than about 6.70 nM to less than about 0.23 nM.

227. The isolated antibody of any one of the preceding claims, wherein the isolated antibody has dissociation constant (K_D) for human TREM2-Fc fusion protein that ranges from less than about 0.71 nM to less than about 0.23 nM.
228. The isolated antibody of any one of the preceding claims, wherein the isolated antibody has dissociation constant (Kd) for human monomeric TREM2 protein that ranges from less than about 6.70 nM to less than about 0.66 nM.

229. The isolated antibody of any one of the preceding claims, wherein the isolated antibody has dissociation constant (Kd) for mouse TREM2-Fc fusion protein that ranges from less than about 4.90 nM to less than about 0.35 nM.

230. An isolated nucleic acid encoding the antibody of any one of the preceding claims.

231. A vector comprising the nucleic acid of claim 230.

232. A host cell comprising the vector of claim 231.

233. A method of producing an antibody, comprising culturing the cell of claim 232 so that the antibody is produced.

234. The method of claim 233, further comprising recovering the antibody produced by the cell.

235. A pharmaceutical composition comprising the antibody of any one of claims 1-229 and a pharmaceutically acceptable carrier.

236. A method of preventing, reducing risk, or treating an individual having a disease, disorder, or injury selected from the group consisting of dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, amyotrophic lateral sclerosis, Huntington’s disease, Taupathy disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma, lupus, acute and chronic colitis, wound healing, Crohn’s disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet’s disease, Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear palsy, cortical basal ganglionic degeneration, acute disseminated encephalomyelitis, granulomartous disorders, Sarcoidosis, diseases of aging, seizures, spinal cord injury, traumatic brain injury, age related macular degeneration, glaucoma, retinitis pigmentosa, retinal degeneration, respiratory tract infection, sepsis, eye infection, systemic infection, lupus, arthritis,
multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrosic disease, Paget's
disease of bone, and cancer, comprising administering to the individual a therapeutically
effective amount of an isolated antibody that binds to a TREM2 protein.

237. A method of inducing or promoting innate immune cell survival or wound healing an
individual in need thereof, comprising administering to the individual a therapeutically effective
amount of an isolated agonist antibody that binds to a TREM2 protein.

238. The method of claim 236 or claim 237, wherein the isolated antibody is:
   (a) an agonist antibody;
   (b) an inert antibody; or an
   (c) an antagonist antibody.

239. The method of claim 238, wherein:
   (a) the antibody is of the IgG class the IgM class, or the IgA class; and/or
   (b) the antibody has an IgG1, IgG2, IgG3, or IgG4 isotype

240. The method of claim 239, wherein the antibody comprises one or more amino acid
substitutions in the Fc region are at a residue position selected from the group consisting of:
       M252Y, S254T, T256E, and any combination thereof;
       L235E, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, and any combination thereof;
   (c) L235A, G237A, S228P, L236E, S267E, E318A, L328F, M252Y, S254T, T256E, and
       any combination thereof;
       S254T, T256E, and any combination thereof;
       M252Y, S254T, T256E, and any combination thereof; or
       M252Y, S254T, T256E, N297A, N297Q, and any combination thereof,
wherein the numbering of the residues is according to EU numbering.

241. The method of ant one of claims 236-240, wherein the isolated antibody:

(a) binds to one or more amino acids within amino acid residues 43-50 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 43-50 of SEQ ID NO: 1; or

(b) one or more amino acids within amino acid residues 49-57 of SEQ ID NO: 1, or amino acid residues on a TREM2 protein corresponding to amino acid residues 49-57 of SEQ ID NO: 1.

242. The method of ant one of claims 236-241, wherein the isolated antibody:

(a) binds essentially the same TREM2 epitope as the antibody Ab52;

(b) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and/or HVR-H3 of the monoclonal antibody Ab52; and/or wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and/or HVR-L3 of the monoclonal antibody Ab52;

(c) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:398, or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:398, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:399 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:399, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:400 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:400, and/or wherein the light chain variable domain comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:401 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:401, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:402 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:402, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:403 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:403;

(d) binds essentially the same TREM2 epitope as the antibody Ab21;
(e) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and/or HVR-H3 of the monoclonal antibody Ab21; and/or wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and/or HVR-L3 of the monoclonal antibody Ab21; or

(f) comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:404 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:404, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:405 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:405, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:406 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:406, and/or wherein the light chain variable domain comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:407 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:407, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:408 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:408, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:409 or an amino acid sequence with at least about 95% homology to the amino acid sequence of SEQ ID NO:409.

243. The method of claim 236, wherein the isolated antibody is the isolated antibody of any one of claims 1-229.

244. The method of claim 237, wherein the isolated agonist antibody is the isolated antibody of any one of claims 1-83, 118-190, and 217-229.

245. The method of any one of claims 236 and 238-243, wherein the individual has a heterozygous variant of TREM2, wherein the variant comprises one or more substitutions selected from the group consisting of:

i. a glutamic acid to stop codon substitution in the nucleic acid sequence encoding amino acid residue Glu14 of SEQ ID NO: 1;
ii. a glutamine to stop codon substitution in the nucleic acid sequence encoding amino acid residue Gln33 of SEQ ID NO: 1;

iii. a tryptophan to stop codon substitution in the nucleic acid sequence encoding amino acid residue Trp44 of SEQ ID NO: 1;

iv. an arginine to histidine amino acid substitution at an amino acid corresponding to amino acid residue Arg47 of SEQ ID NO: 1;

v. a tryptophan to stop codon substitution in the nucleic acid sequence encoding amino acid residue Trp78 of SEQ ID NO: 1;

vi. a valine to glycine amino acid substitution at an amino acid corresponding to amino acid residue Val126 of SEQ ID NO: 1;

vii. an aspartic acid to glycine amino acid substitution at an amino acid corresponding to amino acid residue Asp134 of SEQ ID NO: 1; and

viii. a lysine to asparagine amino acid substitution at an amino acid corresponding to amino acid residue Lys186 of SEQ ID NO: 1.

The method of any one of claims 236, 238-243, and 245, wherein the individual has a heterozygous variant of TREM2, wherein the variant comprises a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G313 of the nucleic acid sequence encoding SEQ ID NO: 1; a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G267 of the nucleic acid sequence encoding SEQ ID NO: 1; or both.

The method of any one of claims 236, 238-243, and 245-246, wherein the individual has a heterozygous variant of DAP12, wherein the variant comprises one or more variants selected from the group consisting of:

i. a methionine to threonine substitution at an amino acid corresponding to amino acid residue Met1 of SEQ ID NO: 2;

ii. a glycine to arginine amino acid substitution at an amino acid corresponding to amino acid residue Gly49 of SEQ ID NO: 2;

iii. a deletion within exons 1-4 of the nucleic acid sequence encoding SEQ ID NO: 2;

iv. an insertion of 14 amino acid residues at exon 3 of the nucleic acid sequence encoding SEQ ID NO: 2; and
v. a guanine nucleotide deletion at a nucleotide corresponding to nucleotide residue G141 of the nucleic acid sequence encoding SEQ ID NO: 2.

248. The method of any one of claims 236 and 238-243, wherein the cancer is selected from the group consisting of bladder cancer, brain cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, kidney cancer, renal cell cancer, renal pelvis cancer, leukemia, lung cancer, melanoma, non-Hodgkin’s lymphoma, pancreatic cancer, prostate cancer, ovarian cancer, fibrosarcoma, and thyroid cancer.

249. The method of any one of claims 236, 238-243, and 248, further comprising administering to the individual at least one antibody that specifically binds to an inhibitory checkpoint molecule, and/or another standard or investigational anti-cancer therapy.

250. The method of claim 249, wherein the at least one antibody that specifically binds to an inhibitory checkpoint molecule is administered in combination with the isolated antibody.

251. The method of claim 249 or claim 250, wherein the at least one antibody that specifically binds to an inhibitory checkpoint molecule is selected from the group consisting of an anti-PD-L1 antibody, an anti-CTLA4 antibody, an anti-PD-L2 antibody, an anti-PD-1 antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, and anti-HVEM antibody, an anti-B- and T-lymphocyte attenuator (BTLA) antibody, an anti-Killer inhibitory receptor (KIR) antibody, an anti-GAL9 antibody, an anti-TIM3 antibody, an anti-A2AR antibody, an anti-LAG-3 antibody, an anti-phosphatidylyserine antibody, an anti-CD27 antibody, and any combination thereof.

252. The method of claim 249, wherein the standard or investigational anti-cancer therapy is one or more therapies selected from the group consisting of radiotherapy, cytotoxic chemotherapy, targeted therapy, imatinib (Gleevec®), trastuzumab (Herceptin®), adoptive cell transfer (ACT), chimeric antigen receptor T cell transfer (CAR-T), vaccine therapy, and cytokine therapy.

253. The method of any one of claims 236, 238-243, and 248, further comprising administering to the individual at least one antibody that specifically binds to an inhibitory cytokine.
254. The method of claim 253, wherein the at least one antibody that specifically binds to an inhibitory cytokine is administered in combination with the isolated antibody.

255. The method of claim 253 or claim 254, wherein the at least one antibody that specifically binds to an inhibitory cytokine is selected from the group consisting of an anti-CCR2 antibody, an anti-CSF-1 antibody, an anti-IL-2 antibody, and any combination thereof.

256. The method of any one of claims 236, 238-243, and 248, further comprising administering to the individual at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein.

257. The method of claim 256, wherein the at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein is administered in combination with the isolated antibody.

258. The method of claim 256 or claim 257, wherein the at least one agonistic antibody that specifically binds to a stimulatory checkpoint protein is selected from the group consisting of an agonist anti-CD40 antibody, an agonist anti-OX40 antibody, an agonist anti-ICOS antibody, an agonist anti-CD28 antibody, an agonist anti-CD137/4-1BB antibody, an agonist anti-CD27 antibody, an agonist anti-glucocorticoid-induced TNFR-related protein GITR antibody, and any combination thereof.

259. The method of any one of claims 236, 238-243, and 248, further comprising administering to the individual at least one stimulatory cytokine.

260. The method of claim 259, wherein the at least one stimulatory cytokine is administered in combination with the isolated antibody.

261. The method of claim 259 or claim 260, wherein the at least one stimulatory cytokine is selected from the group consisting of TNF-α, IL-10, IL-6, IL-8, CRP, TGF-beta members of the chemokine protein families, IL20 family member, IL-33, LIF, OSM, CNTF, TGF-beta, IL-11, IL-12, IL-17, IL-8, CRP, IFN-α, IFN-β, IL-2, IL-18, GM-CSF, G-CSF, and any combination thereof.
ABSTRACT

The invention is generally directed to methods and compositions that include antibodies, e.g., monoclonal, chimeric, humanized antibodies, antibody fragments, etc., that specifically bind a TREM2 protein, e.g., a mammalian TREM2 and/or human TREM2. The methods provided herein find use in preventing, reducing risk, or treating an individual having dementia, frontotemporal dementia, Alzheimer’s disease, vascular dementia, mixed dementia, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, Huntington's disease, Nasu-Hakola disease, stroke, acute trauma, chronic trauma, lupus, acute and chronic colitis, wound healing, Crohn's disease, inflammatory bowel disease, ulcerative colitis, obesity, Malaria, essential tremor, central nervous system lupus, Behcet's disease, Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy, Shy-Drager syndrome, progressive supranuclear palsy, cortical basal ganglionic degeneration, acute disseminated encephalomyelitis, granulomatous disorders, Sarcoidosis, diseases of aging, seizures, spinal cord injury, traumatic brain injury, age related macular degeneration, glaucoma, retinitis pigmentosa, retinal degeneration, respiratory tract infection, eye infection, systemic infection, lupus, arthritis, multiple sclerosis, low bone density, osteoporosis, osteogenesis, osteopetrotic disease, Paget's disease of bone, or cancer.
FIG. 1A

1  MEPLRLILLLFVTELSGAHNTTIVGQAVGQSLQVSCPYDSDKHKWGRKKWCRCQLGEGKPC  60
+ PL LL+LLF  + +  V Q VAGQ+LV C Y  +K WC++  C
6  LHPLLLLLLLFPGSQAQS-KEVQLQSVAGQTLTVRCQYPPTGLYKEKWCCEASAL-VC  63

61  QRVVSTTHNLWLLSSLRRWNGSTAITDDTLGGLTLTITLRNFLQPHDAGLYQCQLHGSFADT 120
R+V++  ++  W I DD G T+T+ +L+ D+G Y C+ S+
64  IRLVTSSKPRMA----WTSFRTIWDDPDAGFFTIVMTDLREEDSGHYWCRICYRPSDNSV  119

121  LRKVLVEVLADP  132  TREM-2  Human
+ V  ++  P
120  SKSVRFYLVVSP  131  NCTR2_HUMAN
FIG. 2A

sp|Q9N2C2|TREM2_HUMAN Triggering receptor expressed on myeloid cells 2 OS=Homo sapiens GN=TREM2 PE=1 SV=1
Sequence ID: lcl|35715 Length: 230 Number of Matches: 1
Range 1: 6 to 110

<table>
<thead>
<tr>
<th>Score</th>
<th>Expect</th>
<th>Method</th>
<th>Identities</th>
<th>Positives</th>
<th>Gaps</th>
<th>Frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.7 bits(99) 1e-09() Compositional matrix adjust.</td>
<td>34/107(32%)</td>
<td>49/107(45%)</td>
<td>4/107(3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Features:

|    | 9 | LLNHLFYSLEALKTLTEKERLYELKEQGDYKCDYLTEKPASSQKAH---QIIRDGEMPKT 66 |
|    | 6 | LLNHLFVSLEALKTLTEKERLYELKEQGDYKCDYLTEKPASSQKAH---QIIRDGEMPKT 66 |
| 67 | 6 | LACTERPSSHPVQVGR113EDYH8GLLRVMVNLQV64EDSLGYYC 113 |
|    | 64 | VSTHHNWLHLRWRNGSTA110DDLEGTLTITLRLNLQPDAGLYQC |

Attorney Docket No.: 735023000600
FIG. 2B

Ab1 – Heavy chain variable region (SEQ ID NO:242)
EVQLVESGGGLVQPGSSRLSLCAGGRFTSYYYSVWQRQAPGKGLEWSVISGSSTTYYVADSVKGRFTISRDNSKNTLYLQMNLSRAEDTAVYYCAKGTPTLLEQHHWGWGQTVSS

Ab2 – Heavy chain variable region (SEQ ID NO:244)
EVQLVESGGGLVQPGSSRLSLCAGGRFTSYYYSVWQRQAPGKGLEWSVISGSSTTYYVADSVKGRFTISRDNSKNTLYLQMNLSRAEDTAVYYCASKPSYDWSGYSNYYYMDWVGKTGTVSS

Ab3 – Heavy chain variable region (SEQ ID NO:246)
QGQLVQVSGAEVKPGSSRKVSICAGGRFTSYYYSVWQRQAPGKGLEWSVISGSSTTYYVADSVKGRFTISRDNSKNTLYLQMNLSRAEDTAVYYCAREQMVGMDWVGKTGTVSS

Ab4 – Heavy chain variable region (SEQ ID NO:248)
QGQLVQVSGAEVKPGSSRKVSICAGGRFTSYYYSVWQRQAPGKGLEWSVISGSSTTYYVADSVKGRFTISRDNSKNTLYLQMNLSRAEDTAVYYCARGVDSDMYWGWGQTVSS

Ab5 – Heavy chain variable region (SEQ ID NO:250)
QGQLVQVSGAEVKPGSSRKVSICAGGRFTSYYYSVWQRQAPGKGLEWSVISGSSTTYYVADSVKGRFTISRDNSKNTLYLQMNLSRAEDTAVYYCARGSDSYPYVFDIWGWQTVSS

Ab6 – Heavy chain variable region (SEQ ID NO:252)
QGQLVQVSGAEVKPGSSRKVSICAGGRFTSYYYSVWQRQAPGKGLEWSVISGSSTTYYVADSVKGRFTISRDNSKNTLYLQMNLSRAEDTAVYYCARGSDSYPYVFDIWGWQTVSS

Ab7 – Heavy chain variable region (SEQ ID NO:254)
QGQLVQVSGAEVKPGSSRKVSICAGGRFTSYYYSVWQRQAPGKGLEWSVISGSSTTYYVADSVKGRFTISRDNSKNTLYLQMNLSRAEDTAVYYCARGSDSYPYVFDIWGWQTVSS

Ab8 – Heavy chain variable region (SEQ ID NO:258)
QLQIQCQSGPGLVTKSETLSTCTTVGGISSSYYWGWRQPPKGKGLEWGSSSYSGSTYYNPSLKSRTITSVDTSKQFSLKLSSVTAADTAvVYCAARGYRLIIAGMDWGWGQTVSS

Ab9 – Heavy chain variable region (SEQ ID NO:258)
EVQLVQVSGAEVKPGESLKISCKGSYGSTSYWIGWVRQMPKGKGLEWMGIYPGSDDTTTSPFQLQTVTSADKISTAYLQWSSKASDTAMYCAREHISGEVNWTFDPWGWGQTVSS

Ab10 – Heavy chain variable region (SEQ ID NO:260)
EVQLVQVSGAEVKPGESLKISCKGSYGSTSYWIGWVRQMPKGKGLEWMGIYPGSDDTTYSFQLQTVTSADKISTAYLQWSSKASDTAMYCAREAGYLYGELAFDREWGWGQTVSS
FIG. 2B (Cont.)

Ab11 – Heavy chain variable region (SEQ ID NO:262)
EVQLVQSGAEVKKPGKSLKCSCKGKSYYTNYWIGWVRQMPGKGLEWMGIHYPGDSQTRYSFQPFGQVTISADKSIAYLQWSSLKASDTAMYycARAGHY
DGQHLGMDYWGQGTTTVSS

Ab12 – Heavy chain variable region (SEQ ID NO:264)
EVQLVQSGAEVKKPGKSLKCSCKGKSYYTNYWIGWVRQMPGKGLEWMGIHYPGDSQTRYSFQPFGQVTISADKSIAYLQWSSLKASDTAMYycARLGHY
SGTVSSYGMGYWGQGTTTVSS

Ab13 – Heavy chain variable region (SEQ ID NO:266)
EVQLVQSGAEVKKPGKSLKCSCKGKSYYTNYWIGWVRQAPGQGKGLEWGMISAYNQNTYAQIKLGFRVMTTDTSTTSTAYMELRSRLSDDTTAVYYCAR
PSHYDLAWGQQTLTVSS

Ab14 – Heavy chain variable region (SEQ ID NO:268)
EVQLVQESGPGLVKPSITLSLCTTVSGGSISSGYYYSYWWIRQHPGKGLEWIGNIYYSSTYVYNPLKRSVTISVYDTSKNNQFSLKLSVTADAVYVYCARGLYG
YGVLYDVGQGTTTVSS

Ab15 – Heavy chain variable region (SEQ ID NO:268)
EVQLVQESGPGLVKPSITLSLCTTVSGGSISSGYYYSYWWIRQHPGKGLEWIGNIYYSSTYVYNPLKRSVTISVYDTSKNNQFSLKLSVTADAVYVYCARGLYG
YGVLYDVGQGTTTVSS

Ab16 – Heavy chain variable region (SEQ ID NO:271)
QIQLVQESGPGLVKPSITLSLCTTVSGGSISSNNSYWWIRQPPGKGLEWIGNIYYSSTYVYNPLKRSVTISVYDTSKNNQFSLKLSVTADAVYVYCARGLYG
GVFDYWGQGTLTVSS

Ab17 – Heavy chain variable region (SEQ ID NO:271)
QIQLVQESGPGLVKPSITLSLCTTVSGGSISSNNSYWWIRQPPGKGLEWIGNIYYSSTYVYNPLKRSVTISVYDTSKNNQFSLKLSVTADAVYVYCARGLYG
GVFDYWGQGTLTVSS

Ab18 – Heavy chain variable region (SEQ ID NO:274)
QIQLVQESGPGLVKPSITLSLCTTVSGGSISSYYWWSIRQPPGKGLEWIGNIYYSSTYVYNPLKRSVTISVYDTSKNNQFSLKLSVTADAVYVYCARDGGGGEY
SGTPFDIWGQGTTTVSS

Ab19 – Heavy chain variable region (SEQ ID NO:274)
QIQLVQESGPGLVKPSITLSLCTTVSGGSISSYYWWSIRQPPGKGLEWIGNIYYSSTYVYNPLKRSVTISVYDTSKNNQFSLKLSVTADAVYVYCARDGGGGEY
SGTPFDIWGQGTTTVSS

Ab20 – Heavy chain variable region (SEQ ID NO:277)
QIQLVQESGPGLVKPSITLSLCTTVSGGSISSYYWWSIRQPPGKGLEWIGNIYYSSTYVYNPLKRSVTISVYDTSKNNQFSLKLSVTADAVYVYCARSGMASFE
DYWGQGTLTVSS
FIG. 2B (Cont.)

Ab21 – Heavy chain variable region (SEQ ID NO:414)
EVQLVQSGAEVKPKGPESLKISCKGSQGYFSETTYWGWVRQMPGKGLEWMGLIYPGDSDTTRYSFPSOQGVGTISADKSISTAYLQWSSLKASDTAMYYCARAGHYDGGLHGMDCWGQGTTVTSS

Ab22 – Heavy chain variable region (SEQ ID NO:262)
EVQLVQSGAEVKPKGPESLKISCKGSQGYFSETTYWGWVRQMPGKGLEWMGLIYPGDSDTTRYSFPSOQGVGTISADKSISTAYLQWSSLKASDTAMYYCARAGHYDGGLHGMDCWGQGTTVTSS

Ab23 – Heavy chain variable region (SEQ ID NO:280)
EVQLLESGGGLVQPGGSLRLSCAASGETFSSYAAMSWVRQAPKGLEWVSAISGSGGSTYYADSVKGRTITRDNSKNTLYIQMNSLRAEDTAVYYCAKIGGHSMDVWGQGTVTSS

Ab24 – Heavy chain variable region (SEQ ID NO:282)
EVQLLESGGGLVQPGGSLRLSCAASGETFSSYAAMSWVRQAPKGLEWVSAISGSGGSTYYADSVKGRTITRDNSKNTLYIQMNSLRAEDTAVYYCAKPLKRGFRGFPWGQGTVTSS

Ab25 – Heavy chain variable region (SEQ ID NO:284)
EVQLLESGGGLVQPGGSLRLSCAASGETFSSYAAMSWVRQAPKGLEWVSVISGSGGSTYYADSVKGRTITRDNSKNTLYIQMNSLRAEDTAVYYCAKEDGRTTMDVGQGTVTSS

Ab26 – Heavy chain variable region (SEQ ID NO:286)
EVQLLESGGGLVQPGGSLRLSCAASGETFSSYAAMSWVRQAPKGLEWVSVISGSGGSTYYADSVKGRTITRDNSKNTLYIQMNSLRAEDTAVYYCAKDOYSVLDYWQGQGTVTSS

Ab27 – Heavy chain variable region (SEQ ID NO:288)
EVQLLESGGGLVQPGGSLRLSCAASGETFSSYAAMSWVRQAPKGLEWVSAISGSGGSTYYADSVKGRTITRDNSKNTLYIQMNSLRAEDTAVYYCAKISREGVYEDYWQGQGTVTSS

Ab28 – Heavy chain variable region (SEQ ID NO:290)
EVQLLESGGGLVQPGGSLRLSCAASGETFSSYAAMSWVRQAPKGLEWVSAISGSGGSTYYADSVKGRTITRDNSKNTLYIQMNSLRAEDTAVYYCARGAVGARIHYFEDYWQGQGTVTSS

Ab29 – Heavy chain variable region (SEQ ID NO:292)
QVQLVESGGGVQPGGSLRLSCAASGETFSSYGMHFWVRQAPKGLEWVAVISYDGSNKYYADSVKGRTITRDNSKNTLYIQMNSLRAEDTAVYYCARGQYYGGSWFPDPWGQGTVTSS

Ab30 – Heavy chain variable region (SEQ ID NO:294)
EVQLLESGGGLVQPGGSLRLSCAASGETFSSYAAMSWVRQAPKGLEWVSAISGSGGSTYYADSVKGRTITRDNSKNTLYIQMNSLRAEDTAVYYCARGQEVAYEQHGWGQGTVTSS
FIG. 2B (Cont.)

Ab31 – Heavy chain variable region (SEQ ID NO:296)
QVQLVESGGGLVQPGSRLSLCAASGFTTSSYAMSWVRQAPGKELEWMALWYDGSNKYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
DGYYDEVEDHGWQGTMVTVSS

Ab32 – Heavy chain variable region (SEQ ID NO:298)
EVLQLESGGGLLQPGSRLSLCAASGFTTSSYAMSWVRQAPGKELEWVASISGSGGTYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
PKHIYYLVLDYWQQGTLVTVSS

Ab33 – Heavy chain variable region (SEQ ID NO:300)
QVQLVESGGGVVQPGSRLSLCAASGFTTSSYAMSWVRQAPGKELEWVAVISYDGSNKYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
AGGHLFDYWQQGTLVTVSS

Ab34 – Heavy chain variable region (SEQ ID NO:302)
QVQLVESGGGLVQPGSRLSLCAASGFTTSSYAMSWVRQAPGKELEWVAVISYDGSNKYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
DGGEYYYDVFDIYWQQGTMVTVSS

Ab35 – Heavy chain variable region (SEQ ID NO:304)
EVLQLESGGGLLQPGSRLSLCAASGFTTSSYAMSWVRQAPGKELEWVASISGSGGTYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
TRTSGYGASNYYFDYWQQGTLVTVSS

Ab36 – Heavy chain variable region (SEQ ID NO:306)
QVQLVESGGGVVQPGSRLSLCAASGFTTSTYAMSWVRQAPGKELEWVAVIWYDGSNKYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
TGTTAAASPAFDIYWQQGTMVTVSS

Ab37 – Heavy chain variable region (SEQ ID NO:308)
EVLQLESGGGLLQPGSRLSLCAASGFTTSSYAMSWVRQAPGKELEWVASISGSGGTYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
VYMLGMDVWGQGTTVTVSS

Ab38 – Heavy chain variable region (SEQ ID NO:310)
QVQLVESGGGVVQPGSRLSLCAASGFTTSTYAMSWVRQAPGKELEWVAVIWYDGSNKYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
APVDYGGEYPEYFQHWQQGTLVTVSS

Ab39 – Heavy chain variable region (SEQ ID NO:312)
EVLQLESGGGLLQPGSRLSLCAASGFTTSSYAMSWVRQAPGKELEWVASISGSGGTYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAKHYI
HGIALFDIYWQQGTMVTVSS

Ab40 – Heavy chain variable region (SEQ ID NO:304)
EVLQLESGGGLLQPGSRLSLCAASGFTTSSYAMSWVRQAPGKELEWVASISGSGGTYYADSVKGRFTISRDSNKNTLYLQMNSLRAEDTAVVYCAR
TRTSGYGASNYYFDYWQQGTLVTVSS
FIG. 2B (Cont.)

Ab41 – Heavy chain variable region (SEQ ID NO:315)  
EVQLESGGGLVQPGGLSLRLSCASGGTFTSFYAMSVVRQAPGKPLEWVSAIGSASGGTYADSVKGRFTISRDSKNTLYQMNLSRAEDTAVYCARAMA
  RKSVAFDIQWGQGTMVTSS

Ab42 – Heavy chain variable region (SEQ ID NO:317)  
EVQLESGGGLVQPGGLSLRLSCASGGTFTSFSSAMSVVRQAPGKPLEWVSAIGSASGGTYADSVKGRFTISRDSKNTLYQMNLSRAEDTAVYCAKVPYS
  QRGTAEDPQWGQGTLTVSS

Ab43 – Heavy chain variable region (SEQ ID NO:319)  
EVQLESGGGLVQPGGLSLRLSCASGGTFTSFSSAMSVVRQAPGKPLEWVSAIGSASGGTYADSVKGRFTISRDSKNTLYQMNLSRAEDTAVYCAKSPAV
  AGYRADYWGQGTLTVSS

Ab44 – Heavy chain variable region (SEQ ID NO:306)  
QVQLVESGGGVQPGSRSLRLSCASGGTFTSSYGMHWWVRQAPGKPLEWVAVIWDGSNKYYADSVKGRFTISRDSKNTLYQMNLSRAEDTAVYCARGT
  GAAAAASPAFDIQWGQGTMVTSS

Ab45 – Heavy chain variable region (SEQ ID NO:322)  
QVQLVESGAEVKKPAGSVKVSCKASGSTTSSYHFWVRQAPGKPLEWVMIINPISISYAIKQFQGRVTMTRTDSTSTVYMELSSLRSEDATAVYCARGP
  GYTTYLDDYMDVWGQGTLTVSS

Ab46 – Heavy chain variable region (SEQ ID NO:324)  
QVQLVESGAEVKKPAGSVKVSCKASGSTTSSYHFWVRQAPGKPLEWVMIINPISISYAIKQFQGRVTMTRTDSTSTVYMELSSLRSEDATAVYCARPA
  KTADYWQGQGTIVSS

Ab47 – Heavy chain variable region (SEQ ID NO:326)  
QVQLVESGAEVKKPAGSVKVSCKASGSTTSSYHFWVRQAPGKPLEWVMIINPISISYAIKQFQGRVTMTRTDSTSTVYMELSSLRSEDATAVYCARPG
  KSMDVWGQGTTTVSS

Ab48 – Heavy chain variable region (SEQ ID NO:326)  
QVQLVESGAEVKKPAGSVKVSCKASGSTTSSYHFWVRQAPGKPLEWVMIINPISISYAIKQFQGRVTMTRTDSTSTVYMELSSLRSEDATAVYCARPG
  KSMDVWGQGTTTVSS

Ab49 – Heavy chain variable region (SEQ ID NO:324)  
QVQLVESGAEVKKPAGSVKVSCKASGSTTSSYHFWVRQAPGKPLEWVMIINPISISYAIKQFQGRVTMTRTDSTSTVYMELSSLRSEDATAVYCARPA
  KTADYWQGQGTIVSS

Ab50 – Heavy chain variable region (SEQ ID NO:250)  
QVQLVESGAEVKKPAGSVKVSCKASGSTTSSYHFWVRQAPGKPLEWVMIINPISISYAIKQFQGRVTMTRTDSTSTVYMELSSLRSEDATAVYCARPA
  ESPYTVFDIQWGQGTMVTSS
Ab51 – Heavy chain variable region (SEQ ID NO:331)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMGIINPSPGGSITYAQFKQGRVTMRVDSTTSTTVYMESSLRLRSEDVV YCAARGV
GGQDYDDYMDVWGKGTTVTSS

Ab52 – Heavy chain variable region (SEQ ID NO:415)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMGIINPSPGGSITYAQFKQGRVTMRVDSTTSTTVYMESSLRLRSEDVV YCASEAD
DSSGYFLGLDVGQGTMTVTS

Ab53 – Heavy chain variable region (SEQ ID NO:250)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMGIINPSPGGSITYAQFKQGRVTMRVDSTTSTTVYMESSLRLRSEDVV YCARAPQ
ESPYVFDIWGQGTMTVTS

Ab54 – Heavy chain variable region (SEQ ID NO:322)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMGIINPSPGGSITYAQFKQGRVTMRVDSTTSTTVYMESSLRLRSEDVV YCARGP
GYTTALDDYMDVWGKGTTVTSS

Ab55 – Heavy chain variable region (SEQ ID NO:335)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMGINPNSGGSITYAQFKQGRVTMRVDSTTSTTVYMESSLRLRSDTAVYCAR
PLYHPMIFDYWGQGTMTVTS

Ab56 – Heavy chain variable region (SEQ ID NO:337)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMGINPNSGGSITYAQFKQGRVTMRVDSTTSTTVYMESSLRLRSDTAVYCAR
SSVNWGQGTLTVTS

Ab57 – Heavy chain variable region (SEQ ID NO:339)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMNWASYNGNTNYAQKLOQRVTMRVDSTTSTTVYMESSLRLRSDTAVYCAR
PTKAYGSGSIVDFPWQGTMTVTS

Ab58 – Heavy chain variable region (SEQ ID NO:341)
EVQLVQSGAELKPGESLCKSCGRYSFTWSYWGQWVRQPMGKLEWGMGIIPYSDTRYSFPSFQGVTSADKSIYALQWSSLKASDTAMYCARLGYS
TGATAFVGWQGTMVTS

Ab59 – Heavy chain variable region (SEQ ID NO:343)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMGINPNSGGSITYAQFKQGRVTMRVDSTTSTTVYMESSLRLRSDTAVYCAR
GVWYSLFDIWGQGTMTVTS

Ab60 – Heavy chain variable region (SEQ ID NO:345)
QVQLVQSGAELKPGASVKVSCCKASGYTFTGYSFTYWQAPGQGLEWGMGINPNSGGSITYAQFKQGRVTMRVDSTTSTTVYMESSLRLRSDTAVYCAR
SKMGDDWGQGTMTVTS
FIG. 2B (Cont.)

Ab61 – Heavy chain variable region (SEQ ID: NO:347)
QVQLVQSGAEVKPGASVTKASCLSYTFTSYGNYHGWRQAPGQGLEWMGWSAYGNTNYAQKL queries GVRVPRVSYPFQHQWQQGTLTVSS

Ab62 – Heavy chain variable region (SEQ ID: NO:349)
EQLVQSGAEVKPGSGLKISCCKGSGYSESTSYWGWVRQMPGKGLEWMGIIYPDSDTRYSPSQFQQTISADKSIAYLQWSLKLASDTAMYYCARAGHY
DDWSGLGLDVWQDGQTMVTSS

Ab63 – Heavy chain variable region (SEQ ID: NO:351)
QVQLVQSGAEVKPGASVTKASCLSYTFTSYGNYHGWRQAPGQGLEWMGWSAYGNTNYAQKL queries GVRVPRVSYPFQHQWQQGTLTVSS

Ab64 – Heavy chain variable region (SEQ ID: NO:353)
EQLVQSGAEVKPGSGLKISCCKGSGYSESTSYWGWVRQMPGKGLEWMGIIYPDSDTRYSPSQFQQTISADKSIAYLQWSLKLASDTAMYYCARI GRW
SSGSTAFDIWGQDGQTMVTSS

Ab65 – Heavy chain variable region (SEQ ID: NO:355)
EQLVQSGAEVKPGSGLKISCCKGSGYSESTSYWGWVRQMPGKGLEWMGIIYPDSDTRYSPSQFQQTISADKSIAYLQWSLKLASDTAMYYCARI GRK
PPSGVAFDIWGQDGQTMVTSS

Ab66 – Heavy chain variable region (SEQ ID: NO:357)
QVQLVQSGAEVKPGASVTKASCLSYTFTSYGNYHGWRQAPGQGLEWMGWINPSGCTNYAQKFPQGRVMTMRTDSISTAYLQWSLKLASDTAMYYCARA
GHIKTHVDWGQDGQTMVTSS

Ab67 – Heavy chain variable region (SEQ ID: NO:326)
QVQLVQSGAEVKPGASVTKASCLSYTFTSYGNYHGWRQAPGQGLEWMGWINPSGCTTYAQKFPQGRVMTMRTDSISTAYLQWSLKLASDTAMYYCARP
KSMDIYWGQGTTVSS

Ab68 – Heavy chain variable region (SEQ ID: NO:360)
QVQLVQSGAEVKPGASVTKASCLSYTFTSYGNYHGWRQAPGQGLEWMGWINPSGCTNYAQKFPQGRVMTMRTDSISTAYLQWSLKLASDTAMYYCARA
SMTDYWGQGTTLTVSS

Ab69 – Heavy chain variable region (SEQ ID: NO:362)
QVQLVQSGAEVKPGASVTKASCLSYTFTSYGNYHGWRQAPGQGLEWMGWINPSGCTNYAQKFPQGRVMTMRTDSISTAYLQWSLKLASDTAMYYCARA
KSVDIHYWGQGTTLTVSS

Ab70 – Heavy chain variable region (SEQ ID: NO:345)
QVQLVQSGAEVKPGASVTKASCLSYTFTSYGNYHGWRQAPGQGLEWMGWINPSGCTNYAQKFPQGRVMTMRTDSISTAYLQWSLKLASDTAMYYCARA
SKMGDDIYWGQGTTLTVSS
FIG. 2B (Cont.)

Ab71 – Heavy chain variable region (SEQ ID NO:365)
QVQLVQSGAEVKKPGASVVKVSCKASGYTFTGSGYHMHWVRQAPGQPQGLEWMGIINPSGGSTSYAQQPGRVTMTRDTSTVYMELSLRSQEDTAYYCAARDCS
THDYDIAEDLWGGTQVTSS

Ab72 – Heavy chain variable region (SEQ ID NO:367)
QVQLVQSGAEVKKPGESLKLSCCKSGYSTFSTSYWIGWVRQMPGKGLEWMGIYPPGDSDTLYSPFSFQGVTISADKSIAYLQWSLKLASDTAMYCARAKML
DDGAYFEDLWGGTQVTSS

Ab73 – Heavy chain variable region (SEQ ID NO:369)
QVQLVQSGAEVKKPGESLKLSCCKSGYSTFSTSYWIGWVRQMPGKGLEWMGIYPPGDSDTLYSPFSFQGVTISADKSIAYLQWSLKLASDTAMYCARAKML
DDGAYFEDLWGGTQVTSS

Ab74 – Heavy chain variable region (SEQ ID NO:357)
QVQLVQSGAEVKKPGASVVKVSCKASGYTFTGSGYHMHWVRQAPGQPQGLEWMGIINPSGGSTSYAQQPGRVTMTRDTSTVYMELSLRSQEDTAYYCAAR
DHYDIAEDLWGGTQVTSS

Ab75 – Heavy chain variable region (SEQ ID NO:372)
QVQLVQSGAEVKKPGASVVKVSCKASGYTFTGSGYHMHWVRQAPGQPQGLEWMGIINPSGGSTSYAQQPGRVTMTRDTSTVYMELSLRSQEDTAYYCAAR
DHYDIAEDLWGGTQVTSS

Ab76 – Heavy chain variable region (SEQ ID NO:374)
QVQLVQSGAEVKKPGASLRLSCAASTGFESSYWSMNWVRQAPGPKLGEWVSISSSSYITYADSVKGRFTISRDNKSLYQMSLIRAEATDNYCARGGGR
RGDNNWTDPPWGGTQVTSS

Ab77 – Heavy chain variable region (SEQ ID NO:376)
QVQLVQSGAEVKKPGASLRLSCAASTGFESSYWSMNWVRQAPGPKLGEWVAISYDGSKYYADSVKGRFTISRDNKSLYQMSLIRAEATDNYCARGGGR
RGDNNWTDPPWGGTQVTSS

Ab78 – Heavy chain variable region (SEQ ID NO:378)
QVQLVQSGAEVKKPGASLRLSCAASTGFESSYWSMNWVRQAPGPKLGEWVAISYDGSKYYADSVKGRFTISRDNKSLYQMSLIRAEATDNYCARGGGR
RGDNNWTDPPWGGTQVTSS

Ab79 – Heavy chain variable region (SEQ ID NO:380)
QVQLVQSGAEVKKPGASLRLSCAASTGFESSYWSMNWVRQAPGPKLGEWVSISGSSSTITYADSVKGRFTISRDNKSLYQMSLIRAEATDNYCARGGGR
RGDNNWTDPPWGGTQVTSS

Ab80 – Heavy chain variable region (SEQ ID NO:382)
QVQLVQSGAEVKKPGASLRLSCAASTGFESSYWSMNWVRQAPGPKLGEWVSISGSSSTITYADSVKGRFTISRDNKSLYQMSLIRAEATDNYCARGGGR
RGDNNWTDPPWGGTQVTSS
FIG. 2B (Cont.)

Ab81 – Heavy chain variable region (SEQ ID NO:384)
QVQLVQSGAEVKPGSSVKVSCKASGTVTSSYYASWVRQAPGQGLEWWMCGIIPITFGTANYAAQPKFQRGVTITLDESTSTAYMELSSLRSEDFTAVYCYCARDSGN
YDYWSGALRSYGQGTLTVSS

Ab82 – Heavy chain variable region (SEQ ID NO:386)
QVQLQESGPGGLVQPSQTLSLTCTVSGGISISSGGYYWSWRQHPKGLEIGYIYYNGSTYYNSNLKSRTISVDTSKQFLKLSVTAADTAAYCYCARYVSSS
WYKAWGQGTMVTTVSS

Ab83 – Heavy chain variable region (SEQ ID NO:388)
QVQLQQQWAGDLLKPSETLSLCTAVYGGFISHGGYYSWIRQPPPGKGLEIGFEIDHSOSSKYNPSLAKSTISVDTSKQFLKLSVTAADTAAYCARYGVEY
GRPGYSAFIDWGGQGTMVTTVSS

Ab84 – Heavy chain variable region (SEQ ID NO:351)
QVQLVQSGAEVKPGASVKVSCKASGTVTSSYYASWVRQAPGQGLEWMMGWIYNGNTNYAQQKGLQGRVTTDDTISTYAMELRSSLRSSDFTAVYCYCARG
SGSYDSWYDQGQGTMVTTVSS

Ab85 – Heavy chain variable region (SEQ ID NO:391)
QVQLQVESGPGGVQPSQRLSLSCAASGFTESSYGMHSWWVRQAPGKGLELWAVIYDYDSNKKYAAVDSVEGRTISRDNSKNTLQLMNSLRAEDTAAYCAYKD
LOGYYYGAAAYGMDWGGQGTVTSS

Ab86 – Heavy chain variable region (SEQ ID NO:393)
QVQLQVESGPGGVQPSQRLSLSCAASGFTESSYGMHSWWVRQAPGKGLELWAVISYDYDSNKKYAAVDSVEGRTISRDNSKNTLQLMNSLRAEDTAAYCAYDG
VYYGLGNWDPWGQGTMVTTVSS

Ab87 – Heavy chain variable region (SEQ ID NO:395)
QVQLQGESGPGGLVQPSQTLSLTCTVSGGISISSGGYYWSWRQPPPGKGLEIGYIYYNGSTYNPSLAKSTISVDTSKQFLKLSVTAADTAAYCARYHGWRD
GWDPWGQGTMVTTVSS
FIG. 2C

Ab1 – Light chain variable region (SEQ ID NO:243)
EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPQAPRLIIYGASTRATGIPARFSGSGSGETFTLTISESSLQSEDFAVYYCQQLPYWPPTFGGGTKVEIK

Ab2 – Light chain variable region (SEQ ID NO:245)
EIVL.TQSPATLSVSPGERATLSCRASQSVSSQLAWYQQKPQAPRLIIYGASTRATGIPARFSGSGSGETFTLTISESSLQSEDFAVYYCQYFFYWPPTFGGGTKVEIK

Ab3 – Light chain variable region (SEQ ID NO:247)
DIQMSTQPSSSLASVGDRVITTCQASQDTISNYLNWYQQKPQAKPKLIIYDASNLATGIPSRFSGSGSGETFTLTISESSLQPEDIAITYYCQQPENFYPFPTFGGGTKVEIK

Ab4 – Light chain variable region (SEQ ID NO:249)
EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPQAPRLIIYGASTRATGIPARFSGSGSGETFTLTISESSLQSEDFAVYYCQODHDYPFTFGGGTKVEIK

Ab5 – Light chain variable region (SEQ ID NO:251)
EIVMTQSPGTLSSVSPGERATLSCRASQSVSSYLYAWYQQKPQAPRLIIYGASSRATGIPDASFSGSGSGETFTLTISRLPTedFAVYYCQYFFSPFTFGGGTKVEIK

Ab6 – Light chain variable region (SEQ ID NO:253)
EIVL.TQSPATLSVSPGERATLSCRASQSVSSYLYAWYQQKPQAPRLIIYDASKRATGIPARFSGSGSGETFTLTISWLPLEPDAVYYCQQRVNVLPPTFGGGTKVEIK

Ab7 – Light chain variable region (SEQ ID NO:255)
EIVL.TQSPATLSVSPGERATLSCRASQSVSSYLYAWYQQKPQAPRLIIYDASKRATGIPARFSGSGSGETFTLTISWLPLEPDAVYYCQQRISYPTFGGGTKVEIK

Ab8 – Light chain variable region (SEQ ID NO:257)
DIQMSTQPSSSLASVGDRVITTCRASQISSYLNWYQQKPQAKPKLIIYGASSLQSGVSRFSGSGSGETFTLTISESSLQPEDAFAYYCQIDDTPTFGGGTKVEIK

Ab9 – Light chain variable region (SEQ ID NO:259)
EIVL.TQSPATLSVSPGERATLSCRASQSVSSYLYAWYQQKPQAPRLIIYDASNRATGIPARFSGSGSGETFTLTISWLPLEPDAVYYCQFQSYWPWPTFGGGTKVEIK

Ab10 – Light chain variable region (SEQ ID NO:261)
EIVL.TQSPGTLSSVSPGERATLSCRASQSVSSYLYAWYQQKPQAPRLIIYGASSRATGIPDASFSGSGSGETFTLTISRLPLEPDAVYYCQOHDDSPPTFGGGTKVEIK
FIG. 2C (Cont.)

Ab11 – Light chain variable region (SEQ ID NO:263)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSDYLYWYQQKPGQAPRLLIYGA\r\rASSRATGIPDRFSGSGTGDTFTLTISRL\r\rLEPDFAVYCYCQDISYPTFGGGTKVEIK

Ab12 – Light chain variable region (SEQ ID NO:265)
DIQMTQSPSSILASVGR\r\rVTITCRASQNISSNYL\r\rwryQQKP\r\rGKPAPLLIYDA\r\r\rSNNRTGIPARFSGSGTGDTFTLTISSLEPDFAVYCCQVSNY\r\rp\r\rFTFGGGTKVEIK

Ab13 – Light chain variable region (SEQ ID NO:267)
EIVLTQSPATLSPLSGERATLSCRASQSVSSDYLYWYQQKPGQAPRLLIYDA\r\rSNNRTGIPARFSGSGTGDTFTLTISSLEPDFAVYCCQVSNYPTFGGGTKVEIK

Ab14 – Light chain variable region (SEQ ID NO:269)
DIQMTQSPSSILASVGR\r\rVTITC\r\rASQNISSNYL\r\rwryQQKP\r\rGKPAPLLIYDA\r\r\rSNNRTGIPARFSGSGTGDTFTLTISSLEPDFAVYCCQVSNYPTFGGGTKVEIK

Ab15 – Light chain variable region (SEQ ID NO:270)
DIQMTQSPSSILASVGR\r\rVTITC\r\rASQNISSNYL\r\rwryQQKP\r\rGKPAPLLIYDA\r\r\rSNNRTGIPARFSGSGTGDTFTLTISSLEPDFAVYCCQVSNYPTFGGGTKVEIK

Ab16 – Light chain variable region (SEQ ID NO:272)
DIQMTQSPSSILASVGR\r\rVTITC\r\rASQNISSNYL\r\rwryQQKP\r\rGKPAPLLIYDA\r\r\rSNNRTGIPARFSGSGTGDTFTLTISSLEPDFAVYCCQVSNYPTFGGGTKVEIK

Ab17 – Light chain variable region (SEQ ID NO:273)
DIQMTQSPSSILASVGR\r\rVTITC\r\rASQNISSNYL\r\rwryQQKP\r\rGKPAPLLIYDA\r\r\rSNNRTGIPARFSGSGTGDTFTLTISSLEPDFAVYCCQVSNYPTFGGGTKVEIK

Ab18 – Light chain variable region (SEQ ID NO:275)
DIQMTQSPSSILASVGR\r\rVTITC\r\rASQNISSNYL\r\rwryQQKP\r\rGKPAPLLIYDA\r\r\rSNNRTGIPARFSGSGTGDTFTLTISSLEPDFAVYCCQVSNYPTFGGGTKVEIK

Ab19 – Light chain variable region (SEQ ID NO:276)
DIQMTQSPSSILASVGR\r\rVTITC\r\rASQNISSNYL\r\rwryQQKP\r\rGKPAPLLIYDA\r\r\rSNNRTGIPARFSGSGTGDTFTLTISSLEPDFAVYCCQVSNYPTFGGGTKVEIK

Ab20 – Light chain variable region (SEQ ID NO:278)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSDYLYWYQQKPGQAPRLLIYGA\r\rASSRATGIPDRFSGSGTGDTFTLTISRL\r\rLEPDFAVYCYCQDISYPTFGGGTKVEIK
FIG. 2C (Cont.)

Ab21 – Light chain variable region (SEQ ID NO:416)
EIVMTQSPGTLSPGERALTSRASQVSYYLYAALWYQQPKPQAPRLLYGASRNATGIPDRLFSGSNGSGLTFTLTLSRLPEFADVYYCQQDDSSAPYTFFGGTTKVEIK

Ab22 – Light chain variable region (SEQ ID NO:279)
EIVMTQSPGTLSPGERATLSRASQVSYYLYAALWYQQPKPQAPRLLYGASSRTAGIPDRLFSGSNGSGLTFTLTLSRLPEFADVYYCQQDDDRSPYTFFGGTTKVEIK

Ab23 – Light chain variable region (SEQ ID NO:281)
DIVMTQSPDSLAVSLGERATINCKSSQSYLYSSSNKNYLWYQQPKPQPPKLLISWASTRESGVPDFSGSGSGLTFTLTLSSLQAEDVAVYYCQQAYLPITFGGGTKVEIK

Ab24 – Light chain variable region (SEQ ID NO:283)
DIQMTQSPSSLASVGDRVTITCRASQISSYLYNYWYQQPKPQAPRLLYAASLQSGVPSRFGSNSGLTFTLTLSSLQPEDATYLYCQQAFSPPPWTFGGTTKVIEIK

Ab25 – Light chain variable region (SEQ ID NO:285)
EIVLTQSPGTLSLSPGERATLSRASQVSYYLYAALWYQQPKPQAPRLLYGASSRTAGIPDRLFSGSNGSGLTFTLTLSRLPEFADVYYCQQDDRSPTTFGGTTKVIEIK

Ab26 – Light chain variable region (SEQ ID NO:287)
EIVLTQSPATLSPGERATLSRASQVSYYLYAALWYQQPKPQAPRLLYDASNNRTAGIPARSGSNGSGLTFTLTLSSLQPEDAVYYCQQEDLPIFTFGGTKVEIK

Ab27 – Light chain variable region (SEQ ID NO:289)
EIVLTQSPATLSPGERATLSRASQVSYYLYAALWYQQPKPQAPRLLYDASNNRTAGIPARSGSNGSGLTFTLTLSSLQPEDAVYYCQQYNENPPIFTFGGTKVEIK

Ab28 – Light chain variable region (SEQ ID NO:291)
EIVLTQSPATLSPGERATLSRASQSYSLYALWYQQPKPQAPRLLYGASKRTAGIPARSGSNGSGLTFTLTLSSLQPEDAFYYCQQYRLRPTTFFGGTTKVIEIK

Ab29 – Light chain variable region (SEQ ID NO:293)
EIVLTQPSGTLSLSPGERATLSRASQSYSLYALWYQQPKPQAPRLLYGASSRTAGIPDRLFSGSNGSGLTFTLTLSRLPEFADVYYCQQPGAPVTTFGGTTKVIEIK

Ab30 – Light chain variable region (SEQ ID NO:295)
DIQMTQSPSSLASVGDRVTITCRASQISSYLYNYWYQQPKPQPPKLLYGAASLQSGVPSRFGSNSGLTFTLTLSSLQPEDATYLYCQQYTTPTTFGGTTKVIEIK
FIG. 2C (Cont.)

Ab41 – Light chain variable region (SEQ ID NO:316)
EIVLTVSPATLSLPGERATLSCRASQSVSSYLAHWYQQKPGQAPRLIYDASNRATGIPARFSGSQSGTDFTLTISLLEPEDFTVYYCQQRYALPITFPGGSGKV
EIK

Ab42 – Light chain variable region (SEQ ID NO:318)
EIVLTVSPGTLSLPGERATLSCRASQSVSSYLAHWYQQKPGQAPRLIYGASSRATGIPDRIFSGSFGSGTDFTLTISRLEPEDFAVYYCCQYASPPITFPGGSGKV
EIK

Ab43 – Light chain variable region (SEQ ID NO:320)
DIQMTVSPSSLASVGDRTITCRASQISRNYLHWYQQKPGKAPKLLIIYAASSLQGVPFRSFGSGTDFTLTISLLEPEDFATYYCQQYVYSTPITFPGGSGKV
EIK

Ab44 – Light chain variable region (SEQ ID NO:321)
EIVMVTQSPATLSLPGERATLSCRASQSVSSYLAHWYQQKPGQAPRLIIYDSSNRATGIPARFSGSQSGTDFTLTISLLEPEDFAVYYCQOLVHIPPTFPGGSGKV
EIK

Ab45 – Light chain variable region (SEQ ID NO:323)
EIVMVTQSPATLSVSIPGERTLSCRASQSVSSPLAWYQQKPGQAPRLIIYGASTRATGIPARFSGSQSGTIFETLTISSLQSEDFAVYYCQQLDDWIFTFPGGSGKV
EIK

Ab46 – Light chain variable region (SEQ ID NO:325)
EIVLTVSPATLSLPGERATLSCRASQSVSSYLAHWYQQKPGQAPRLIIYDSSNRATGIPARFSGSQSGTDFTLTISLLEPEDFAVYYCQQYNSPYTIFPGGSGKV
EIK

Ab47 – Light chain variable region (SEQ ID NO:327)
EIVLTVSPGTLSLPGERATLSCRASQSVSSYLAHWYQQKPGQAPRLIIYDASNRATGIPARFSGSQSGTDFTLTISLLEPEDFAVYYCQQRLYPTIFPGGSGKV
EIK

Ab48 – Light chain variable region (SEQ ID NO:328)
EIVLTVSPATLSLPGERATLSCRASQSVSSYLAHWYQQKPGQAPRLIIYDASNRATGIPARFSGSQSGTDFTLTISLLEPEDFAVYYCQQARAPITFPGGSGKV
EIK

Ab49 – Light chain variable region (SEQ ID NO:329)
EIVLTVSPATLSLPGERATLSCRASQSVSSYLAHWYQQKPGQAPRLIIYDASKRATGIPARFSGSQSGTDFTLTISLLEPEDFAVYYCQQRTSHPTFPGGSGKV
EIK

Ab50 – Light chain variable region (SEQ ID NO:330)
EIVLTVSPGTLSLPGERATLSCRASQSVSSYLAHWYQQKPGQAPRLIIYGAASSRATGIPDRIFSFGSGTDFTLTISRLEPEDFAVYYCQQYAGSPITFPGGSGKV
EIK
FIG. 2C (Cont.)

Ab51 – Light chain variable region (SEQ ID NO:332)
DIQMTQSPSSLSASVGDRVITITCRASQSSISYLNWYQQKPGKAPKLLIYAASSLQSGVPQSFSGSGSDFTTLTISSLQPEDFATYYCQHFDDVFTFGGTKV
EIK

Ab52 – Light chain variable region (SEQ ID NO:417)
EIYMTQSPATLSVPGERATLSCRAQSVSSYLAWYQQKPGQAPRLIYGAAGTRATGIAVPARFSGSGSDFITTLTISSLQFDAVYCYCQQVNSLPPFTFGGT
EIK

Ab53 – Light chain variable region (SEQ ID NO:333)
EIYLTQSPGTLSVPGERATLSCRAQSVSSYLAWYQQKPGQAPRLIYGAASRATGIPDRFSGSGSDFTTLTISSLQPEDFATYYCQNYVNSPFTFGGT
EIK

Ab54 – Light chain variable region (SEQ ID NO:334)
DIQMTQSPSSLSASVGDRVITITCRASQSSISYLNWYQQKPGKAPKLLIYAASSLQSGVPQSFSGSGSDFTTLTISSLQPEDFATYYCQSSDDPPTFGGT
EIK

Ab55 – Light chain variable region (SEQ ID NO:336)
EIYLTQSPATLSVPGERATLSCRAQSVSSYLAWYQQKPGQAPRLIYDAANRATGIPARFSGSGSDFTTLTISSLQPEDFAVYCYCQQLSTYPLTFGGGT
EIK

Ab56 – Light chain variable region (SEQ ID NO:338)
EIYLTQSPATLSVPGERATLSCRAQSVSSYLAWYQQKPGQAPRLIYDAANRATGIPARFSGSGSDFTTLTISSLQPEDFAVYCYCQQSSVYPTTFGGGT
EIK

Ab57 – Light chain variable region (SEQ ID NO:340)
EIYLTQSPATLSVPGERATLSCRAQSVSSYLAWYQQKPGQAPRLIYDASKRATGIPARFSGSGSDFTTLTISSLQPEDFAYCYCQQVSLEPLTFGGTK
EIK

Ab58 – Light chain variable region (SEQ ID NO:342)
DIQMTQSPATLSASVGGDRVTITCRASQSSISWLNWYQQKPGKAPKLLIYDASSLQSGVPQSFSGSGSDFTEFTLTISSLQPEDATYCLDYNSSPITFGGT
EIK

Ab59 – Light chain variable region (SEQ ID NO:344)
DIQMTQSPSSLSASVGDRVTITCTASQDISYLNWYQQKPGKAPKLLIYDAANLETGVPQSFSGSGSDFTFFTISSLQPEPAAYCYCQHIALPFTFGGT
EIK

Ab60 – Light chain variable region (SEQ ID NO:346)
EIYLTQSPATLSVPGERATLSCRAQSVSSYLAWYQQKPGQAPRLIYDASKRATGIPARFSGSGSDFTTLTISSLQPEDFAVYCYCQRAASPITFGGT
EIK
FIG. 2C (Cont.)

Ab61 – Light chain variable region (SEQ ID NO:348)
EIVLTQSPATLSLPGERATLSCRASQSVSYLAWYQQKPGQAPRLIIYDSSNRATGIPARFSGSGSTDFALTISSLEPEDFAVYYCQQAFNRPTTFGGGTKVEIK

Ab62 – Light chain variable region (SEQ ID NO:350)
EIVLTQSPATLSLPGERATLSCRASQSVSYLAWYQQKPGQAPRLIIYDASKRATGIPARFSGSGSTDFALTISSLEPEDFAVYYCQQSVHPYTFGGGTKVEIK

Ab63 – Light chain variable region (SEQ ID NO:352)
DIQMTQSPSSVSASVGDRVTITCRASQGIDSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSTDFALTISSLQPEDFATYYCQQAYSLPPTTFGGGTKVEIK

Ab64 – Light chain variable region (SEQ ID NO:354)
EIVMTQSPATLSVPGERATLSCRASQSVSSNLAWYQQKPGQAPRLIIYGASTRATGIPARFSGSGSTTFALTISSLQSEDFAVYYCQQDDDDGYTFGGGTKVEIK

Ab65 – Light chain variable region (SEQ ID NO:356)
EIVLTQSPATLSLPGERATLSCRASQSVSYLAWYQQKPGQAPRLIIYDASNRATGIPARFSGSGSTDFALTISSLEPEDFAVYYCQQDDDDGYTFGGGTKVEIK

Ab66 – Light chain variable region (SEQ ID NO:358)
EIVLTQSPGTLSLPGERATLSCRASQSVSYLAWYQQKPGQAPRLIIYDASNRATGIPARFSGSGSTDFALTISSLEPEDFAVYYCQQRSAAYPTTFGGGTKVEIK

Ab67 – Light chain variable region (SEQ ID NO:359)
EIVLTQSPATLSLPGERATLSCRASQSVSYLAWYQQKPGQAPRLIIYDASNRATGIPARFSGSGSTDFALTISSLEPEDFAVYYCQQRSHPPTTFGGGTKVEIK

Ab68 – Light chain variable region (SEQ ID NO:361)
EIVLTQSPATLSLPGERATLSCRASQSVSYLAWYQQKPGQAPRLIIYDASNRATGIPARFSGSGSTDFALTISSLEPEDFAVYYCQQRANYPTTFGGGTKVEIK

Ab69 – Light chain variable region (SEQ ID NO:363)
EIVLTQSPATLSLPGERATLSCRASQSVSYLAWYQQKPGQAPRLIIYDASNRATGIPARFSGSGSTDFALTISSLEPEDFAVYYCQRADYPTTFGGGTKVEIK

Ab70 – Light chain variable region (SEQ ID NO:364)
EIVLTQSPATLSLPGERATLSCRASQSVSYLAWYQQKPGQAPRLIIYDASNRATGIPARFSGSGSTDFALTISSLEPEDFAVYYCQRSVYPTTFGGGTKVEIK
Ab71 – Light chain variable region (SEQ ID NO:366)
EVMTQSPGTLSSLPGERATLSGRASQVSSYLYLAWYQKPGQQPRLLIIYGASNRATGIPDRFSGSGSTDFTLTISRLQEDFAVYYCQQAAGSHPETFGGGTVKVEIK

Ab72 – Light chain variable region (SEQ ID NO:368)
DIQMTQSPSSSASVGDRTVITTCQASQDFTNYLNYQKPGKAPKLIIYDANILETGVPSPRSSRSGSTDFTLTISSIQLPEDIATYYCQQDVYNYPPTTFGGGTVKVEIK

Ab73 – Light chain variable region (SEQ ID NO:370)
EVMTQSPATLSLSGGERATLSGRASQVSSYLYLAWYQKPGQAPRLLIIYGASNRATGIPARPSGSGSTDFTLTISISSLEPEDFAVYYCQQDDNYPPYTTFGGGTVKVEIK

Ab74 – Light chain variable region (SEQ ID NO:371)
EVMTQSPATLSLSGGERATLSGRASQVSSYLYLAWYQKPGQAPRLLIIYGASNRATGIPARPSGSGSTDFTLTISISSLEPEDFAVYYCQQRSTFTPFTTFGGGTVKVEIK

Ab75 – Light chain variable region (SEQ ID NO:373)
EVMTQSPATLSLSGGERATLSGRASQVSSYLYLAWYQKPGQAPRLLIIYGASNRATGIPARPSGSGSTDFTLTISISSLEPEDFAVYYCQQSNYPPFTTFGGGTVKVEIK

Ab76 – Light chain variable region (SEQ ID NO:375)
DIVMTQSPDSLAVSLGERATINCQKSSQSLYSSNKNKYLYLAWYQKPGQPPKLLIIYGASTRESGVPDREFSGSGSTDFTLTISSIQLAEVYVYYCQYHDAPPTTFGGGTVKVEIK

Ab77 – Light chain variable region (SEQ ID NO:377)
DIVMTQSPDSLAVSLGERATINCQKSSQSLYSSNKNKYLYLAWYQKPGQPPKLLIIYGASTRESGVPDREFSGSGSTDFTLTISSIQLAEVYVYYCQYAVVVPPTTFGGGTVKVEIK

Ab78 – Light chain variable region (SEQ ID NO:379)
EVMLQSPATLSSLPGERATLSGRASQVSSYLYLAWYQKPGQAPRLLIIYGASNRATGIPARPSGSGSTDFTLTISISSLEPEDFAVYYCQQADNWPFETFGGGTVKVEIK

Ab79 – Light chain variable region (SEQ ID NO:381)
DIVMTQSPSLPVTGPASICRSRSSLHISNGNYLQDLYLQKQGSPQPLLIYGSHRASGVPDREFSGSGSTDFTLKISRVEAEVDVGVYYCMQALESPTTFGGGTVKVEIK

Ab80 – Light chain variable region (SEQ ID NO:383)
EVILQSPATLSSLPGERATLSGRASQVSSYLYLAWYQKPGQAPRLLIIYGASNRATGIPARPSGSGSTDFTLTISISSLEPEDFAVYYCQYVNWPFETFGGGTVKVEIK
FIG. 2C (Cont.)

Ab81 – Light chain variable region (SEQ ID NO:385)
EIVLTQSPATLSLSPGERATLSCRASQSVSYLAWYQQKPGQAPRLIYDASNRTAGIPARFSGSGSTDFTLTISSLQEPEDFAVYYCQQSNWPWTFGGGTKVEIK

Ab82 – Light chain variable region (SEQ ID NO:387)
DIQMTQSPSSVSASVGDRVVTITCRASQGISSWALAWYQQKPGKAPKLIIYAASSLQSGVPSRFSGSGSTDFTLTISSLQEDFAYYCQQASTFPTTFGGGTKVEIK

Ab83 – Light chain variable region (SEQ ID NO:389)
DIQMTQSPSSVSASVGDRVVTITCRASQGISSWALAWYQQKPGKAPKLIIYAASSLQSGVPSRFSGSGSTDFLTISLLQEDFATYYCQQRNSLPLFGGGTKVEIK

Ab84 – Light chain variable region (SEQ ID NO:390)
DIQMTQSPSSLSASVGDRVVTITCRASQGISSWALAWYQQKPGKAPKLIIYAASSLQSGVPSRFSGSGSTDFLTISLLQEDFATYYCQSYDEPITTFGGGTKVEIK

Ab85 – Light chain variable region (SEQ ID NO:392)
DIQMTQPSSTLSASVGDRVVTITCRASQGISSWALAWYQQKPGKAPKLIIYAASSLQSGVPSRFSGSGSTDFLTISLLQEDFATYYCQQEYDYPPLTFGGGTKVEIK

Ab86 – Light chain variable region (SEQ ID NO:394)
DIQMTQSPSTLSSASVGDRVVTITCRASQGISSWALAWYQQKPGKAPKLIIYKAASSLESQGVPFSGGSSTGEFTLTISLLQEDFATYYCQLNSPSTTFGGGTKVEIK

Ab87 – Light chain variable region (SEQ ID NO:396)
EIVLTQSPATLSLSPGERATLSCRASQSVSRAYWYQQKPGQAPRLIYDASNRTAGIPARFSGSGSTDFTLTISSLQEPEDFAVYYCQQYIFWPPTFGGGTKVEIK
FIG. 4B

#88-Isotype

Sec Alone
mAb

#1

#43

#14

#60

#65

#45

#22
FIG. 7B

IP-Syk

<table>
<thead>
<tr>
<th>1</th>
<th>9</th>
<th>14</th>
<th>20</th>
<th>22</th>
<th>45</th>
<th>65</th>
<th>ctr</th>
<th>16</th>
<th>77</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns</td>
<td>5'</td>
<td>5'</td>
<td>5'</td>
<td>5'</td>
<td>5'</td>
<td>5'</td>
<td>5'</td>
<td>5'</td>
<td>5'</td>
</tr>
</tbody>
</table>

pTyr

Syk

Syk phosphorylation

fold change over isotype control

0 | 1 | 9 | 14 | 20 | 22 | 45 | 65 | 16 | 77
FIG. 10A

IP-TREM2

45,655crl

ns 2', 2', 2'

38kDa -

31kDa -

24kDa -

FIG. 10B

IP-TREM-2

WT

Trem-2-/-

1. 9 22 45 crl

ns 2', 2', 2'

38kDa -

31kDa -

24kDa -

pDAP12

DAP12

actin

45,655crl

ns 2', 2', 2'

38kDa -

31kDa -

24kDa -
FIG. 12

Phagocytosis of E. coli-Phrodo/apoptotic cells

BMM plated 20ng/ml MCSF

Add 20ng/ml MCSF

Add 20ng/ml MCSF

E. coli

WT

Trem-2^{−/−}

pHrodo-positive (MFI)

apoptotic cells

WT

Trem-2^{−/−}

BMMmac : apoptotic cells
Application Data Sheet 37 CFR 1.76  

Attorney Docket Number 735023000600  

Application Number  

Title of Invention ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF  

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.78. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.  

Secrecy Order 37 CFR 5.2  

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)  

Inventor Information:  

Inventor 1  

Legal Name  

Prefix Given Name Middle Name Family Name Suffix  
Kate MONROE  

Residence Information (Select One)  

☐ US Residency ☐ Non US Residency ☐ Active US Military Service  

City Berkeley State/Province CA Country of Residence US  

Mailing Address of Inventor:  

Address 1 c/o 953 Indiana Street  
Address 2  

City San Francisco State/Province CA  
Postal Code 94107 Country US  

Inventor 2  

Legal Name  

Prefix Given Name Middle Name Family Name Suffix  
Tina SCHAWBE  

Residence Information (Select One)  

☐ US Residency ☐ Non US Residency ☐ Active US Military Service  

City San Francisco State/Province CA Country US  

Mailing Address of Inventor:  

Address 1 c/o 953 Indiana Street  
Address 2  

City San Francisco State/Province CA  
Postal Code 94107 Country US  

Inventor 3  

Legal Name  

Prefix Given Name Middle Name Family Name Suffix  
Francesca AVOGADRI-CONNORS  

Residence Information (Select One)  

☐ US Residency ☐ Non US Residency ☐ Active US Military Service
<table>
<thead>
<tr>
<th>City</th>
<th>State/Province</th>
<th>Country of Residence</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Mateo</td>
<td>CA</td>
<td>US</td>
</tr>
<tr>
<td>San Francisco</td>
<td>CA</td>
<td>US</td>
</tr>
<tr>
<td>San Francisco</td>
<td>CA</td>
<td>US</td>
</tr>
<tr>
<td>San Francisco</td>
<td>CA</td>
<td>US</td>
</tr>
<tr>
<td>Woodside</td>
<td>CA</td>
<td>US</td>
</tr>
</tbody>
</table>
Title of Invention: ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF

Mailing Address of Inventor:

Address 1: 150 Normandy Lane

City: Woodside
State/Province: CA
Postal Code: 94062
Country: US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.

Correspondence Information:
Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).

☐ An Address is being provided for the correspondence Information of this application.

Customer Number: 20872
Email Address: patentdocket@molo.com

Application Information:

Title of the Invention: ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF
Attorney Docket Number: 735023000600
Small Entity Status Claimed: X
Application Type: Provisional
Subject Matter: Utility
Total Number of Drawing Sheets (if any): 47
Suggested Figure for Publication (if any):

Filing By Reference:
Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., “Domestic Benefit/National Stage Information” and “Foreign Priority Information”).

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Intellectual Property Authority or Country:

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

Request Not to Publish: I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.
Application Data Sheet 37 CFR 1.76

Title of Invention
ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF

Representative Information:
Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:  
- ☐ Customer Number
- ☐ US Patent Practitioner
- ☐ Limited Recognition (37 CFR 11.9)

Customer Number: 20872

Domestic Benefit/National Stage Information:
This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78. When referring to the current application, please leave the application number blank.

Prior Application Status

<table>
<thead>
<tr>
<th>Application Number</th>
<th>Continuity Type</th>
<th>Prior Application Number</th>
<th>Filing Date (YYYY-MM-DD)</th>
</tr>
</thead>
</table>

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.

Foreign Priority Information:
This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX), the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

<table>
<thead>
<tr>
<th>Application Number</th>
<th>Country</th>
<th>Filing Date (YYYY-MM-DD)</th>
<th>Access Code (if applicable)</th>
</tr>
</thead>
</table>

Additional Foreign Priority Data may be generated within this form by selecting the Add button.
Application Data Sheet 37 CFR 1.76

Title of Invention

ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.
### Application Data Sheet 37 CFR 1.76

**Attorney Docket Number:** 735023000600  
**Application Number:**

**Title of Invention:** ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF

#### Applicant

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an assignee under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

<table>
<thead>
<tr>
<th>Assignee</th>
<th>Legal Representative under 35 U.S.C. 117</th>
<th>Joint Inventor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person to whom the inventor is obligated to assign.</td>
<td>Person who shows sufficient proprietary interest</td>
<td></td>
</tr>
</tbody>
</table>

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

**Name of the Deceased or Legally Incapacitated Inventor:**

If the Applicant is an Organization check here. [ ]

<table>
<thead>
<tr>
<th>Prefix</th>
<th>Given Name</th>
<th>Middle Name</th>
<th>Family Name</th>
<th>Suffix</th>
</tr>
</thead>
</table>

#### Mailing Address Information:

**Address 1**

**Address 2**

**City**

**State/Province**

**Country**

**Postal Code**

**Phone Number**

**Fax Number**

**Email Address**

Additional Applicant Data may be generated within this form by selecting the Add button.

#### Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

#### Assignee

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

**Remove**
<table>
<thead>
<tr>
<th>Application Data Sheet 37 CFR 1.76</th>
<th>Attorney Docket Number</th>
<th>735023000600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title of Invention</td>
<td>ANTI-TREM2 ANTIBODIES AND METHODS OF USE THEREOF</td>
<td></td>
</tr>
</tbody>
</table>

If the Assignee or Non-Applicant Assignee is an Organization check here. □

<table>
<thead>
<tr>
<th>Prefix</th>
<th>Given Name</th>
<th>Middle Name</th>
<th>Family Name</th>
<th>Suffix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mailing Address Information For Assignee including Non-Applicant Assignee:

<table>
<thead>
<tr>
<th>Address 1</th>
<th>Address 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>City</th>
<th>State/Province</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Country</th>
<th>Postal Code</th>
<th>Phone Number</th>
<th>Fax Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Email Address

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications

<table>
<thead>
<tr>
<th>Signature</th>
<th>Date (YYYY-MM-DD)</th>
<th>First Name</th>
<th>Last Name</th>
<th>Registration Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>/Roberto K. Rodriguez/</td>
<td>2015-03-18</td>
<td>Roberto K</td>
<td>Rodriguez</td>
<td>66244</td>
</tr>
</tbody>
</table>

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.
The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.
<table>
<thead>
<tr>
<th><strong>Electronic Acknowledgement Receipt</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EFS ID:</strong></td>
</tr>
<tr>
<td><strong>Application Number:</strong></td>
</tr>
<tr>
<td><strong>International Application Number:</strong></td>
</tr>
<tr>
<td><strong>Confirmation Number:</strong></td>
</tr>
<tr>
<td><strong>Title of Invention:</strong></td>
</tr>
<tr>
<td><strong>First Named Inventor/Applicant Name:</strong></td>
</tr>
<tr>
<td><strong>Customer Number:</strong></td>
</tr>
<tr>
<td><strong>Filer:</strong></td>
</tr>
<tr>
<td><strong>Filer Authorized By:</strong></td>
</tr>
<tr>
<td><strong>Attorney Docket Number:</strong></td>
</tr>
<tr>
<td><strong>Receipt Date:</strong></td>
</tr>
<tr>
<td><strong>Filing Date:</strong></td>
</tr>
<tr>
<td><strong>Time Stamp:</strong></td>
</tr>
<tr>
<td><strong>Application Type:</strong></td>
</tr>
</tbody>
</table>

**Payment information:**

<table>
<thead>
<tr>
<th>Submitted with Payment</th>
<th>yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Payment Type</td>
<td>Deposit Account</td>
</tr>
<tr>
<td>Payment was successfully received in RAM</td>
<td>$930</td>
</tr>
<tr>
<td>RAM confirmation Number</td>
<td>8930</td>
</tr>
<tr>
<td>Deposit Account</td>
<td>031952</td>
</tr>
</tbody>
</table>

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

- Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)
- Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)
Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

### File Listing:

<table>
<thead>
<tr>
<th>Document Number</th>
<th>Document Description</th>
<th>File Name</th>
<th>File Size(Bytes)/Message Digest</th>
<th>Multi Part./.zip</th>
<th>Pages (if appl.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Transmittal of New Application</td>
<td>735023000600TranProv.pdf</td>
<td>42369</td>
<td>no</td>
<td>3</td>
</tr>
</tbody>
</table>

### Warnings:

### Information:

| 2               | 735023000600Specification.pdf     | 795934                             | yes                             | 289             |

#### Multipart Description/PDF files in .zip description

<table>
<thead>
<tr>
<th>Document Description</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specification</td>
<td>1</td>
<td>254</td>
</tr>
<tr>
<td>Claims</td>
<td>255</td>
<td>288</td>
</tr>
<tr>
<td>Abstract</td>
<td>289</td>
<td>289</td>
</tr>
</tbody>
</table>

### Warnings:

### Information:

| 3               | Drawings-only black and white line drawings | 735023000600Drawings_Filing.pdf | 5280169 | no | 47 |

### Warnings:

### Information:

| 4               | Application Data Sheet               | 735023000600ADS.pdf              | 1560936 | no | 8  |

### Warnings:

### Information:

| 5               | Fee Worksheet (5806)                 | fee-info.pdf                      | 31863   | no | 2  |

### Warnings:

### Information:

**Total Files Size (in bytes)**: 7711271
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

**New Applications Under 35 U.S.C. 111**
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

**National Stage of an International Application under 35 U.S.C. 371**
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/OE/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

**New International Application Filed with the USPTO as a Receiving Office**
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.