ANTIBODIES TARGETING RANKL AND SCLEROSTIN AND USES THEREOF

[0001] This application claims priority to U.S. Provisional Application No. 63/520,102, filed August 17, 2023, which is incorporated herein by reference in its entirety.

1. Reference to Sequence Listing Submitted Electronically

[0002] This application incorporates by reference a Sequence Listing as an XML file entitled "022A005WO02_SL" created on August 14, 2024 and having a size of 84,092 bytes.

2. Field

[0003] The present invention relates to molecular biology and cell biology. Provided herein include bispecific antibodies that specifically bind to both human RANKL and human sclerostin and uses thereof in, for example, reducing osteoporosis.

3. Background

[0004] Osteoporosis is a debilitating disease in humans characterized by marked decreases in skeletal bone mass and mineral density, structural deterioration of bone including degradation of bone microarchitecture and corresponding increases in bone fragility and susceptibility to fracture in afflicted individuals. Dysregulated function of both osteoclast and osteoblast contribute to osteoporosis. Current strategies for the prevention and treatment of osteoporosis target either osteoclast or osteoblast, which may offer some benefit to individuals but either cannot ensure resolution of the condition or are associated with undesirable side effects. As such, there is an urgent unmet need for additional therapeutic treatment options for osteoporosis. The compositions and methods provided in this disclosure address this need and provide related advantages.

4. Summary

[0005] Provided herein are bispecific antibodies that can simultaneously bind to human RANKL and human sclerostin. The antibodies disclosed herein can effectively block osteoclast differentiation and promote osteoblast functions.

[0006] Provided herein are bispecific antibody comprising (i) a first light chain variable domain (VL1) and a first heavy chain variable domain (VH1), wherein the VL1/VH1 pair specifically binds to human RANKL, and wherein the VL1 comprises VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively, and wherein the VH1 comprises VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5, and 6, respectively; and (ii) a second light chain variable domain (VL2) and a second heavy chain variable domain (VH2), wherein the VL2/VH2 pair specifically binds to human sclerostin; and wherein the

VL2 comprises VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:7, 8, and 9, respectively, and wherein the VH2 comprises VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:10, 11, and 12, respectively. In some embodiments, the VL1, VH1, VL2, and VH2 have the amino acid sequences of SEQ ID NOs:13, 14, 15, and 16, respectively.

[0007] In some embodiments, the bispecific antibodies provided herein comprise (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant domain 1 (CH1), and a Knob-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL2 and VH2, a linker, and a Hole-Fc region.

[0008] In some embodiments, the bispecific antibodies provided herein comprise (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant domain 1 (CH1), and a Hole-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL2 and VH2, a linker, and a Knob-Fc region.

[0009] In some embodiments, the scFv comprises, from N-terminus to C-terminus, VL2, a second linker, and VH2. In some embodiments, the scFv comprises, from N-terminus to C-terminus, VH2, a second linker, and VL2.

[0010] In some embodiments, the bispecific antibodies provided herein comprise (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant domain 1 (CH1), and a Knob-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL1 and VH1, a linker, and a Hole-Fc region.

[0011] In some embodiments, the bispecific antibodies provided herein comprise (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant domain 1 (CH1), and a Hole-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL1 and VH1, a linker and a Knob-Fc region.

[0012] In some embodiments, the scFv comprises, from N-terminus to C-terminus, VL1, a second linker, and VH1. In some embodiments, the scFv comprises, from N-terminus to C-terminus, VH1, a second linker, and VL1.

[0013] In some embodiments of the bispecific antibodies provided herein, the Knob-Fc region is a human IgG2 Fc region variant having up to ten amino acids substitutions, including a T366W substitution; and the Hole-Fc region is a human IgG2 Fc region variant having up to ten amino acids substitutions, including T366S, L368A, Y407V substitutions. In some embodiments, the Knob-Fc region further comprises S354C substitution, and the Hole-Fc region further comprises Y349C substitution. In some embodiments, the Knob-Fc region further comprises Y349C substitution, and the Hole-Fc region further comprises S354C substitution.

[0014] In some embodiments of the bispecific antibodies provided herein, (i) the CL region is kappa CL (Cκ; SEQ ID NO:19) or lambda CL (Cλ; SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions; (ii) the CH1 domain is human IgG2 CH1 domain (SEQ ID NO:36) or a variant thereof having up to ten amino acids substitutions; and/or (iii) the Knob-Fc region and the Hole-Fc region have the amino acid sequences of (1) SEQ ID NOs:29 and 32, respectively, (2) SEQ ID NOs:30 and 33, respectively, or (3) SEQ ID NOs:31 and 34, respectively, or a variant thereof having up to ten amino acids substitutions. In some embodiments, the CL region, CH1 domain, the Knob-Fc region and the Hole-Fc region have the amino acid sequences of (1) SEQ ID NOs:19, 36, 29 and 32, respectively, (2) SEQ ID NOs:19, 36, 30 and 33, respectively, or (3) SEQ ID NOs:19, 36, 31 and 34, respectively.

[0015] In some embodiments of the bispecific antibodies provided herein, the C1, C2, and C3 have amino acid sequences of (1) SEQ ID NOs:51, 53, and 54, respectively, (2) SEQ ID NOs:51, 55, and 56, respectively, (3) SEQ ID NOs:51, 57, and 58, respectively; or (4) SEQ ID NOs:51, 59 and 60, respectively.

[0016] Provided herein are bispecific antibodies comprising a VL1/VH1 pair specifically binds to human RANKL and a VL2/VH2 pair that specifically binds to human sclerostin. In some embodiments, bispecific antibodies provided herein comprise (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1 and a light chain constant region (CL); and (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant region (CH), a linker, and a single chain variable fragment (scFv) comprising VL2 and VH2. In some embodiments, the scFv comprises, from N-terminus to C-terminus, VL2, a second linker, and VH2. In some embodiments, the scFv comprises, from N-terminus to C-terminus, VH2, a second linker, and VL2.

[0017] In some embodiments, bispecific antibodies provided herein comprise (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2 and a light chain constant region (CL); and (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant region (CH), a linker, and a single chain variable fragment (scFv) comprising VL1 and VH1. In some embodiments, the scFv comprises, from N-terminus to C-terminus, VL1, a second linker, and VH1. In some embodiments, the scFv comprises, from N-terminus to C-terminus, VH1, a second linker, and VL1.

[0018] In some embodiments of the bispecific antibodies provided herein: (1) the CL region is kappa CL (Cκ; SEQ ID NO:19) or lambda CL (Cλ; SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions; or (2) the CH region is human IgG1 CH region (SEQ ID NO:21), IgG2 CH region (SEQ ID NO:22), IgG3 CH region (SEQ ID NO:23), or IgG4 CH region (SEQ ID NO:24), or a variant thereof having up to ten amino acids substitutions; or both (1) and (2). In some embodiments, (1) the CL region is kappa CL (Cκ; SEQ ID NO:19); and (2) the CH region is human IgG2 CH region (SEQ ID NO:22).

[0019] In some embodiments, the C1 and C2 have amino acid sequences of (1) SEQ ID NOs:51 and 61, respectively, (2) SEQ ID NOs:51 and 62, respectively. In some embodiments, the C1 and C2 have amino acid sequences of (1) SEQ ID NOs:52 and 63, respectively, (2) SEQ ID NOs:52 and 64, respectively.

[0020] Provided herein are also pharmaceutical compositions comprising a therapeutically effective amount of the bispecific antibody described herein and a pharmaceutically acceptable carrier.

[0021] Provided herein are also polynucleotides encoding a peptide chain of the bispecific antibody described herein. In some embodiments, the polynucleotide provided herein encodes all peptide chains of the bispecific antibody. In some embodiments, provided herein are also a plurality of polynucleotides that collectively encode all peptide chains of the bispecific antibody.

[0022] Provided herein are also vectors comprising the polynucleotide described herein.

[0023] Provided herein are also cell comprising the polynucleotide or plurality of polynucleotides described herein.

[0024] Provided herein are also methods of making a bispecific antibody that specifically binds to human RANKL and human sclerostin comprising culturing the cell described herein under conditions that allow expression of the bispecific antibody.

[0025] Provided herein are also methods of preventing or treating osteoporosis in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the bispecific antibody described herein. In some embodiments, the subject has primary osteoporosis. In some

embodiments, the subject has postmenopausal osteoporosis (Type I). In some embodiments, the subject has senile osteoporosis (Type II). In some embodiments, the subject has secondary osteoporosis. In some embodiments, methods provided herein prevent or treat osteoporosis that is secondary to an endocrine or metabolic cause, a collagen or genetic disorder, a medication cause, or a nutritional cause. In some embodiments, the subject is a human.

[0026] Provided herein are also use of the bispecific antibodies described herein as a medicament. Provided herein are also use of the bispecific antibodies described herein in preventing or treating osteoporosis. Provided herein are also use of the bispecific antibodies described herein for the preparation of a medicament for preventing or treating osteoporosis.

5. Brief Description of Drawings

[0027] **FIGs.1A-1B** provide schematic diagrams of bispecific antibodies provided herein that specifically bind to RANKL and sclerostin ("DRAbs"). **FIG.1A** provides exemplary depictions of the bispecific antibody in knobs-into-holes (KIH) configuration. **FIG.1B** provides an exemplary depiction of the bispecific antibody in IgG-scFv configuration.

[0028] **FIG.2** provides ELISA results showing the specific binding of the DRAbs DR-P7, DR-P15 and DR-P16 to human RANKL (upper panel) and sclerostin (lower panel).

[0029] **FIG.3** provides the results of a TRACP assay demonstrating that the DRAbs DR-P7 and DR-P8 effectively blocked osteoclast differentiation.

[0030] **FIG.4** provides the results of an assay measuring the expression of marker genes (MMP9, FATc1, Acp5, CTSK) for osteoclast differentiation, which demonstrated that the DRAbs DR-P7 and DR-P8 effectively blocked expression of these marker genes.

[0031] **FIG.5** provides the results of a nanoluciferase assay using HEK293 cells, which demonstrated that the DRAbs DR-P7 and DR-P8 effectively blocked sclerostin-mediated inhibition on WNT signaling.

[0032] **FIG.6** provides the results in a mouse model with ovariectomy showing that the DRAb DR-P7 effectively promoted bone re-growth in mice, and synergistic effect was observed with the DRAb as compared to antibody that targeted RANKL or sclerostin alone. OVX: ovariectomy; Tb.N: trabecular number; Tb.Sp: trabecular separation.

6. Detailed Description

[0033] Provided herein are bispecific antibodies that specifically bind to both human RANKL and to human sclerostin ("DRAbs"). Pharmaceutical compositions comprising a therapeutically effective

amount of such antibodies are also disclosed herein. Also disclosed herein are uses of such antibodies and pharmaceutical compositions for reducing osteoporosis and/or for increasing bone mass.

[0034] Osteoporosis is a skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to increased bone fragility and susceptibility to fractures. It is a systemic condition that affects the entire skeleton, resulting in a reduction in bone mineral density and structural integrity. Osteoporosis can be classified into two main types: primary osteoporosis and secondary osteoporosis.

[0035] Primary osteoporosis mainly includes postmenopausal osteoporosis (Type I) or senile osteoporosis (Type II). Postmenopausal osteoporosis (Type I) typically occurs in women after menopause due to a decrease in estrogen levels. Estrogen plays a vital role in maintaining bone density. The loss of estrogen leads to accelerated bone resorption, causing increased bone fragility. Senile osteoporosis (Type II), or age-related osteoporosis affects both men and women as they age. It is a result of the natural aging process and the gradual decline in bone mass that occurs over time. Primary osteoporosis also includes idiopathic osteoporosis, which refers to cases of osteoporosis that occur without an apparent cause. It can affect individuals of any age and may have a genetic component.

[0036] Secondary osteoporosis refers to a condition characterized by decreased bone density and strength as a result of underlying medical conditions or external factors. Several factors can contribute to the development of secondary osteoporosis, including endocrine or metabolic causes, collagen/genetic disorders, medications, and nutritional deficiencies.

[0037] Endocrine or metabolic causes are one of the main categories of secondary osteoporosis. Conditions such as hyperparathyroidism, hyperadrenocorticism, and hyperprolactinemia can disrupt the normal balance of hormones involved in bone remodeling. For example, excessive production of parathyroid hormone in hyperparathyroidism leads to increased bone resorption, resulting in decreased bone mineral density and increased fracture risk.

[0038] Collagen/genetic disorders are another group of conditions associated with secondary osteoporosis. Disorders like Marfan syndrome, osteogenesis imperfecta, Ehlers-Danlos syndrome, glycogen storage diseases, and homocystinuria affect the structure or production of collagen, a key component of bone tissue. Altered collagen synthesis or structure can weaken the bone matrix, making the bones more susceptible to fractures and reducing bone density.

[0039] Certain medications have been linked to secondary osteoporosis due to their effects on bone metabolism. For instance, long-term use of glucocorticoids like prednisone can disrupt the balance between bone formation and resorption. Glucocorticoids inhibit the activity of osteoblasts, leading to

decreased bone formation, while promoting the differentiation and function of osteoclasts, resulting in increased bone resorption. Other medications, such as cyclosporine, methotrexate, and phenobarbital, can also contribute to bone loss and increased fracture risk.

[0040] Nutritional deficiencies and lifestyle factors can also contribute to the development of secondary osteoporosis. Alcoholism, for example, can have a detrimental effect on bone health by interfering with the absorption and metabolism of calcium and other essential nutrients. Chronic liver disease can impair the activation of vitamin D, necessary for calcium absorption. Malabsorption syndromes, where the body is unable to absorb nutrients properly, can lead to inadequate calcium and vitamin D levels, affecting bone health.

[0041] Bone tissue is constantly being remodeled through a dynamic process involving two main cell types: osteoblasts and osteoclasts. Osteoblasts are responsible for bone formation, while osteoclasts are involved in bone resorption. Together, these cells maintain a delicate balance between bone formation and bone resorption, known as bone remodeling. In osteoporosis, this balance is disrupted, leading to an increase in bone resorption and a decrease in bone formation. Osteoclasts become overactive and break down bone at a faster rate than osteoblasts can rebuild it. This results in a net loss of bone mass and a weakening of the skeletal structure.

[0042] Osteoblasts play a crucial role in bone formation. These cells are responsible for synthesizing and depositing new bone matrix, consisting primarily of collagen and other proteins. They also facilitate the mineralization process by promoting the accumulation of calcium and phosphate ions within the bone tissue. When osteoblast activity is impaired or inadequate, as in osteoporosis, compromised bone formation leads to reduced bone density and increased susceptibility to fractures. Osteoblastic functions require the activation of bone morphogenetic protein (BMP), TGFβ, and canonical WNT signaling. Key regulators for WNT signaling include sclerostin, Dickkopf 1 and secreted Frizzled-related protein 1. Estrogen promotes osteogenic differentiation of mesenchymal stem cells. Estrogen also stimulates the production of IGF1 and TGFβ by osteoblasts as well as pro-collagen synthesis. Sclerostin is a protein secreted primarily by osteocytes, which are embedded within the mineralized bone tissue. Its main function is to inhibit the activity of osteoblasts and bone formation. Sclerostin acts by binding to specific receptors on osteoblasts, thereby interfering with the intracellular signaling pathways that promote osteoblast differentiation and function. By inhibiting osteoblast function, sclerostin indirectly affects osteoclast activity and bone resorption.

[0043] Osteoclasts, on the other hand, are multinucleated cells derived from monocytes and are responsible for bone resorption. They secrete enzymes and acids that break down the mineralized

bone matrix, allowing for the release of calcium and other minerals into the bloodstream. In osteoporosis, osteoclasts become hyperactive and excessively resorb bone, further contributing to the loss of bone mass and density. Osteoclast differentiation and bone resorption are controlled by IL-1, TNF-α, TGFβ, M-CSF and RANK-RANKL-osteoprotegerin pathway, in which RANKL (Receptor Activator of Nuclear Factor κB Ligand) plays a pivotal role. RANKL binds to RANK on osteoclast precursor cell and activates MAPK and NF-κB pathway via TRAF6 and TAK1. It promotes the differentiation of osteoclast precursor cells into mature osteoclasts. Once activated, mature osteoclasts are responsible for bone resorption by breaking down the mineralized bone matrix. RANKL stimulates osteoclast formation, activation, and survival, leading to increased bone resorption.

[0044] Osteoporosis treatment includes the use of medications called RANKL inhibitors. These drugs essentially block the interaction between RANKL and its receptor on osteoclasts, thereby preventing the stimulation of osteoclast formation and activity. By decreasing osteoclast-mediated bone resorption, RANKL inhibitors help to preserve bone mass and reduce the risk of fractures in individuals with osteoporosis. Another approach in osteoporosis treatment involves the use of drugs that neutralize or inhibit sclerostin. By blocking the inhibitory effects of sclerostin, these medications allow osteoblasts to function more effectively, promoting bone formation and bone mineral density. Augmenting osteoblast activity can help offset the excessive bone resorption seen in osteoporosis and improve overall bone health.

[0045] Provided in present disclosure are bispecific antibodies that specifically bind to both human RANKL and to human sclerostin ("DRAbs"). By targeting both RANKL and sclerostin, a dual-action approach is achieved, addressing both excessive bone resorption and impaired bone formation seen in osteoporosis. This dual inhibition approach offers the advantage of a more comprehensive intervention, targeting multiple aspects of bone remodeling simultaneously. It not only slows down bone loss but also promotes bone formation, leading to the restoration of bone strength and reducing fracture risk. As shown below, the DRAbs described herein show synergistic effect in enhancing bone formation, which provide greater efficacy in treating osteoporosis compared to targeting either RANKL or sclerostin alone.

[0046] Before the present disclosure is further described, it is to be understood that the disclosure is not limited to the particular embodiments set forth herein, and it is also to be understood that the terminology used herein is for the purpose of describing particular embodiments, and is not intended to be limiting.

6.1 Definitions

[0047] Unless otherwise defined herein, scientific and technical terms used in the present disclosures shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

[0048] The term "a" or "an" entity refers to one or more of that entity; for example, "an antibody," is understood to represent one or more antibodies.

[0049] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0050] As used herein, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects. The term "about" encompasses the exact number recited. In some embodiments, "about" means within plus or minus 10% of a given value or range. In certain embodiments, "about" means that the variation is ±5%, ±4%, ±3%, ±2%, ±1%, ±0.5%, ±0.2%, or ±0.1% of the value to which "about" refers. In some embodiments, "about" means that the variation is ±1%, ±0.5%, ±0.2%, or ±0.1% of the value to which "about" refers.

[0051] The terms "peptide chain," "peptide," "polypeptide," "protein," and their grammatical equivalents as used interchangeably herein refer to polymers of amino acids of any length, which can be linear or branched. It can include unnatural or modified amino acids or be interrupted by non-amino acids. A polypeptide, peptide, polypeptide chain, peptide chain, or protein can also be modified with, for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification.

[0052] The terms "polynucleotide," "nucleic acid," and their grammatical equivalents as used interchangeably herein mean 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.

[0053] The term "variant" as used herein in relation to a protein or a polypeptide with particular sequence features (the "reference protein" or "reference polypeptide") refers to a different protein or polypeptide having one or more (such as, for example, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, or about 1 to about 5) amino acid substitutions, deletions, and/or additions as compared to the reference protein or reference polypeptide. The changes to an amino acid sequence can be amino acid substitutions. The changes to an amino acid sequence can be conservative amino acid substitutions. A functional fragment or a functional variant of a protein or polypeptide maintains the basic structural and functional properties of the reference protein or polypeptide.

[0054] The term "specifically binds," as used herein, means that a polypeptide or molecule interacts more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the epitope, protein, or target molecule than with alternative substances, including related and unrelated proteins. A binding moiety (*e.g.*, antibody) that specifically binds a target molecule (*e.g.*, antigen) can be identified, for example, by immunoassays, ELISAs, Bio-Layer Interferometry ("BLI"), SPR (*e.g.*, Biacore), or other techniques known to those of skill in the art. Typically, a specific reaction will be at least twice background signal or noise and can be more than 10 times background. *See, e.g.*, Paul, ed., 1989, Fundamental Immunology Second Edition, Raven Press, New York at pages 332-336 for a discussion regarding antibody specificity. In some embodiments, "specifically binds" means, for instance, that a binding moiety binds a molecule target with a K_D of about 0.1 mM or less. In some embodiments, "specifically binds" means that a polypeptide or molecule binds a target with a K_D of at about 10 μM or less or about 1 μM or less. In some embodiments, "specifically binds" means that a polypeptide or molecule binds a target with a K_D of at about 0.1 μM or less, about 0.01 μM or less, or about 1 nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include a polypeptide or molecule that recognizes a protein or target in more than one species. Likewise, because of homology within certain regions of polypeptide sequences of different proteins, specific binding can include a polypeptide or molecule that recognizes more than one protein or target.

[0055] The term "binding affinity" as used herein generally refers to the strength of the sum total of noncovalent interactions between a binding moiety and a target molecule (*e.g.*, antigen). The binding of a binding moiety and a target molecule is a reversible process, and the affinity of the binding is typically reported as an equilibrium dissociation constant (K_D). K_D is the ratio of a dissociation rate (k_{off} or k_d) to the association rate (k_{on} or k_a). The lower the K_D of a binding pair, the higher the affinity. A variety of methods of measuring binding affinity are known in the art, any of which can

be used for purposes of the present disclosure. Specific illustrative embodiments include the following. In some embodiments, the "K_D" or "K_D value" can be measured by assays known in the art, for example by a binding assay. The K_D may be measured in a radiolabeled antigen binding assay (RIA) (Chen, *et al.*, (1999) *J. Mol Biol* 293:865-881). The K_D or K_D value can also be measured by using biolayer interferometry (BLI) using, for example, the Gator system (Probe Life), or the Octet-96 system (Sartorius AG). The K_D or K_D value can also be measured by using surface plasmon resonance assays (SPR) by Biacore, using, for example, a BIAcoreTM-2000 or a BIAcoreTM-3000 BIAcore, Inc., Piscataway, NJ). The binding affinity can also be quantified with EC_{50}, which is the concentration of ligand at which half of the target is present in the bound state in a binding assay.

[0056] The terms "identical," percent "identity," and their grammatical equivalents as used herein in the context of two or more polynucleotides or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that can be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two polynucleotides or polypeptides provided herein are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the amino acid sequences that is at least about 10 residues, at least about 20 residues, at least about 40-60 residues, at least about 60-80 residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a target protein or an antibody. In some embodiments, identity exists over a region of the nucleotide sequences that is at least about 10 bases, at least about 20 bases, at least about 40-60 bases, at least about 60-80 bases in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 bases, such as at least about 80-100 bases or more, and in some embodiments the sequences are

substantially identical over the full length of the sequences being compared, such as a nucleotide sequence encoding a protein of interest.

[0057] The term "vector," and its grammatical equivalents as used herein refer to a vehicle that is used to carry genetic material (*e.g.*, a polynucleotide sequence), which can be introduced into a host cell, where it can be replicated and/or expressed. Vectors applicable for use include, for example, expression vectors, plasmids, phage vectors, viral vectors, episomes and artificial chromosomes, which can include selection sequences or markers operable for stable integration into a host cell's chromosome. Additionally, the vectors can include one or more selectable marker genes and appropriate expression control sequences. Selectable marker genes that can be included, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like which are well known in the art. When two or more polynucleotides are to be co-expressed, both polynucleotides can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding polynucleotides can be operationally linked to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The introduction of polynucleotides into a host cell can be confirmed using methods well known in the art. It is understood by those skilled in the art that the polynucleotides are expressed in a sufficient amount to produce a desired product, and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

[0058] As used herein, the term "encode" and its grammatical equivalents refer to the inherent property of specific sequences of nucleotides in a polynucleotide or a nucleic acid, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA can include introns.

[0059] A polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, peptides, proteins,

antibodies, polynucleotides, vectors, cells, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure.

[0060] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 *etc.*, as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[0061] Exemplary genes and polypeptides are described herein with reference to GenBank numbers, GI numbers and/or SEQ ID NOS. It is understood that one skilled in the art can readily identify homologous sequences by reference to sequence sources, including but not limited to GenBank (ncbi.nlm.nih.gov/genbank/) and EMBL (embl.org/).

6.2 Bispecific antibodies targeting human RANKL and human sclerostin

[0062] Provided herein are bispecific antibodies that specifically bind to both human RANKL and to human sclerostin. In some embodiments, the bispecific antibodies provided herein are monoclonal antibodies. In some embodiments, the bispecific antibodies provided herein are isolated. In some embodiments, the bispecific antibodies provided herein are substantially pure.

6.2.1 General

[0063] As used herein and understood in the art, an "antibody" is an immunoglobulin molecule that recognizes and specifically binds a target (*e.g.*, a protein) through at least one antigen-binding fragment which is typically within the variable region of the immunoglobulin molecule. An "antibody" can be of many different types and structures. For example, antibodies can be polyclonal antibodies, monoclonal antibodies, multispecific antibodies, bispecific antibodies, monospecific antibodies, monovalent antibodies, or any other modified immunoglobulin molecule comprising an antigen-binding site. Antibodies also include, but are not limited to, mouse antibodies, camel antibodies, chimeric antibodies, humanized antibodies, and human antibodies. An antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their

heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. Unless expressly indicated otherwise, the term "antibody" as used herein include "antigen-binding fragment" of intact antibodies. The term "antigen-binding fragment" as used herein refers to a portion or fragment of an intact antibody that is the antigenic determining variable region of an intact antibody. Examples of antigen-binding fragments include, but are not limited to, Fab, Fab', F(ab')2, Fv, linear antibodies, single chain antibody molecules (*e.g.*, scFv), heavy chain antibodies (HCAbs), light chain antibodies (LCAbs), disulfide-linked scFv (dsscFv), diabodies, tribodies, tetrabodies, minibodies, dual variable domain antibodies (DVD), single variable domain antibodies (sdAbs; *e.g.*, camelid antibodies, alpaca antibodies), and single variable domain of heavy chain antibodies (VHH).

[0064] As used herein and understood in the art, a "bispecific" antibody is an artificial hybrid antibody having two different antigen binding fragments. The two different antigen binding fragments specifically bind two different target antigens. Bispecific antibodies can be formed from antibody fragments.

[0065] The structure of immunoglobulins has been well characterized (*see*, *e.g.*, FUNDAMENTAL IMMUNOLOGY Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Typically, immunoglobulins comprise two pairs of polypeptide chains, one pair of light (L; low molecular weight) chains and one pair of heavy (H; high molecular weight) chains, all four inter-connected by disulfide bonds.

[0066] Each light chain of an immunoglobulin typically includes a light chain variable region ("VL region") and a light chain constant region ("CL region"). There are two distinct types of light chains, referred to as kappa (κ) of lambda (λ) based on the amino acid sequence of the CL region. The amino acid sequences of the CL regions are well known in the art.

[0067] Each heavy chain typically includes a heavy chain variable region (a "VH region") and a heavy chain constant region (a "CH region"). The VH region can be one of five distinct types, referred to as alpha (α), delta (δ), epsilon (ε), gamma (γ) and mu (μ), based on the amino acid sequence. When combined with a light chain, these distinct types of heavy chains give rise to five well known classes of antibodies, IgA, IgD, IgE, IgG and IgM, respectively. There are four subclasses of IgG, namely, IgG1, IgG2, IgG3 and IgG4. The amino acid sequences of the CH regions of different classes of antibodies are well known in the art.

[0068] The CH region of immunoglobulins comprise more than one domain. For example, the CH region of an IgG antibody is comprised of three domains, heavy chain constant domain 1 (CH1), heavy chain constant domain 2 (CH2), and heavy chain constant domain 3 (CH3). The highly flexible region between the CH1 and CH2 domains is referred to as the "hinge region." Disulfide bonds in the hinge region are part of the interactions between two heavy chains in an

immunoglobulin. The "Fc region" refers to the C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. In IgG, IgA and IgD isotypes, the Fc region is comprised of the CH2 domain and the CH3 domain; IgM and IgE Fc regions contain three heavy chain constant domains (CH domains 2–4). The amino acid sequences of the Fc region of human IgG, IgA, IgD, IgM and IgE, and subtypes IgG1, IgG2, IgG3, and IgG4 are known to those of ordinary skill in the art. As used herein, the Fc region of an IgG heavy chain extends from the hinge region to the carboxyl-terminus of the heavy chain. The native Fc regions can be modified. Modification of the Fc regions are further described below. In some embodiments, a bispecific antibody provided herein can comprise paired Fc domains comprising paired different modifications that promote their association with each other, instead of forming homodimers.

[0069] Unless otherwise stated or contradicted by context, reference to amino acid positions in the constant regions is according to the EU-numbering (Edelman *et al.*, *PNAS.* 1969; 63:78-85, Kabat *et al.*, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, Fifth Edition. 1991 NIH Publication No. 91-3242). A list of exemplary amino acid sequences for constant domains/regions of the human IgG antibodies is provided below. Some exemplary variants are also included, with more variants disclosed in sections below.

[0070] Table 1. Native human IgG constant regions/domains.

Domains/regions	Sequences
Human IgG Cκ	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC (SEQ ID NO:19)
Human IgG Cλ	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPV KAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEK TVAPTECS (SEQ ID NO:20)
Human IgG1 CH	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:21)
Human IgG2 CH	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVE RKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGK EYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:22)
Human IgG3 CH	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRV

	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK (SEQ ID NO:23)
Human IgG4 CH	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:24)
Human IgG1 Fc	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:25)
Human IgG2 Fc	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:26)
Human IgG3 Fc	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK (SEQ ID NO:27)
Human IgG4 Fc	ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:28)
Human IgG1 CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV (SEQ ID NO:35)
Human IgG2 CH1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTV (SEQ ID NO:36)
Human IgG3 CH1	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRV (SEQ ID NO:37)
Human IgG4 CH1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV (SEQ ID NO:38)

[0071] The term "variable region" refers to a portion of the light or heavy chains of an immunoglobulin that is generally located at the amino-terminal of the light or heavy chain and used

in the binding and specificity of each particular antibody for its particular antigen. The variable region of a light chain is referred to as a "light chain variable region" or "VL region," which includes at least one, typically one, "light chain variable domain" or "VL." The variable region of a heavy chain is referred to as a "heavy chain variable region" or "VH region," which includes at least one, typically one, "heavy chain variable domain" or "VH." The variable domains differ extensively in sequence between different antibodies. A "pair of VL and VH" can associate with each other and form a binding site that specifically binds the target antigen or epitope.

[0072] The VH and VL regions can be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). The variability in sequence is concentrated in the CDRs while the less variable portions in the variable domain are referred to as framework regions (FR). The CDRs of the light and heavy chains are primarily responsible for the interaction of the antibody with antigen. Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see also Chothia and Lesk, *J Mol Biol* 1987;196:901-17).

[0073] A CDR refers to one of three hypervariable regions (H1, H2 or H3) within the non-framework region of the immunoglobulin (Ig or antibody) VH β-sheet framework, or one of three hypervariable regions (L1, L2 or L3) within the non- framework region of the antibody VL β-sheet framework. CDR regions are well known to those skilled in the art and have been defined by a variety of methods/systems. These systems and/or definitions have been developed and refined over years and include Kabat, Chothia, IMGT, AbM, and Contact. For example, Kabat defines the regions of most hypervariability within the antibody variable (V) domains (Kabat *et al*, *J. Biol. Chem.* 252:6609-6616 (1977); Kabat, *Adv. Prot. Chem.* 32: 1-75 (1978)). Software programs (*e.g.*, abYsis) are available and known to those of skill in the art for analysis of antibody sequence and determination of CDRs.

[0074] A single chain Fv ("scFv") polypeptide is a covalently linked VL/VH heterodimer which is usually expressed from a gene fusion including VL and VH-encoding genes linked by a peptide-encoding linker. The scFv fragment includes CDRs that are held in appropriate conformation, in particular by using gene recombination techniques. In some embodiments of scFvs, the N-terminus of VL is linked to the C-terminus of the VH via a linker. In some embodiments of scFvs, the N-terminus of VH is linked to the C-terminus of the VL via a linker.

[0075] The term "linker" as used herein refers to one or more amino acid residues inserted between domains (*e.g.*, immunoglobulin domains) to provide sufficient mobility for the domains. A linker can be inserted at the transition between variable domains or between variable and constant domains, respectively, at the sequence level. A person of ordinary skill in the art would understand that the bispecific antibodies and fusion proteins disclosed herein are not limited by the specific linkers exemplified herein. Any peptide linker with the appropriate length and flexibility that allows the VL/VH pair to properly form the antigen-binding site can be used.

[0076] Table 2. Exemplary linkers

Linker	Sequences
Linker 1	(GGGGS)n; n=1, 2, 3, 4, or 5 (SEQ ID NO:41)
Linker 2	(GSGGS)n; n=1, 2, 3, 4, or 5 (SEQ ID NO:42)
Linker 3	(GGGS)n; n=1, 2, 3, 4, or 5 (SEQ ID NO:43)
Linker 4	GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:44)
Linker 5	(EAAAK)n, n=1, 2, 3, 4, or 5 (SEQ ID NO:45)

[0077] The term "humanized antibody" as used herein refers to forms of non-human (*e.g.*, murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Typically, humanized antibodies are human immunoglobulins. In some instances, the variable region residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species. In some instances, residues of the CDRs are replaced by residues from the CDRs of a non-human species (*e.g.*, mouse, rat, hamster, camel) that have the desired specificity, affinity, and/or binding capability. The humanized antibody can be further modified by the substitution of additional residues either in the variable region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or binding capability. The term "human antibody" as used herein refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any of the techniques known in the art.

[0078] Bispecific antibodies provided herein comprise a first VL and VH pair that specifically bind to human RANKL and a second VL and VH pair that specifically binds to human sclerostin. Exemplary VL/VH pairs that specifically bind to human RANKL/sclerostin are provided below. In addition to the specific VL/VH pairs exemplified herein, expressly contemplated also include variants of these VL/VH pairs that retain their bindings to the respective target antigens.

[0079] Table 3A: VL/VH of exemplary anti-RANKL antibodies and CDRs

	VL1	VH1

	EIVLTQSPGTLSLSPGERATLSCR ASQSVRGRYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVFYCQQYGS SPRTFGQGTKVEIK (SEQ ID NO:13)	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSSYAMSWVRQAPGKGLEWVS GITGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAKD PGTTVIMSWFDPWGQGTLVTVSS (SEQ ID NO:14)
CDR1	RASQSVRGRYLA (SEQ ID NO:1)	SYAMS (SEQ ID NO:4)
CDR2	GASSRAT (SEQ ID NO:2)	GITGSGGSTYYADSVKG (SEQ ID NO:5)
CDR3	QQYGSSPRT (SEQ ID NO:3)	DPGTTVIMSWFDP (SEQ ID NO:6)

[0080] Table 3B: VL/VH of exemplary anti-sclerostin antibodies and CDRs

	VL2	**VH2**
	DIQMTQSPSSLSASVGDRVTITCR ASQDISNYLNWYQQKPGKAPKLL IYYTSRLLSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQGDTLP YTFGGGTKVEIK (SEQ ID NO:15)	EVQLVQSGAEVKKPGASVKVSCKA SGYTFTDYNMHWVRQAPGQGLEW MGEINPNSGGAGYNQKFKGRVTMT TDTSTSTAYMELRSLRSDDTAVYYC ARLGYDDIYDDWYFDVWGQGTTVT VSS (SEQ ID NO:16)
CDR1	RASQDISNYLN (SEQ ID NO:7)	DYNMH (SEQ ID NO:10)
CDR2	YTSRLLS (SEQ ID NO:8)	EINPNSGGAGYNQKFKG (SEQ ID NO:11)
CDR3	QQGDTLPYT (SEQ ID NO:9)	LGYDDIYDDWYFDV (SEQ ID NO:12)

[0081] In some embodiments, provided herein are bispecific antibodies comprising (i) a first light chain variable domain (VL1) and a first heavy chain variable domain (VH1), wherein the VL1/VH1 pair specifically binds to human RANKL, and wherein the VL1 comprises VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively, and wherein the VH1 comprises VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5, and 6, respectively; and (ii) a second light chain variable domain (VL2) and a second heavy chain variable domain (VH2), wherein the VL2/VH2 pair specifically binds to human sclerostin; and wherein the VL2 comprises VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:7, 8, and 9, respectively, and wherein the VH2 comprises VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:10, 11, and 12, respectively. In some embodiments of the bispecific antibodies disclosed herein, the VL1 has an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to SEQ ID NO:13. In some embodiments of the bispecific antibodies disclosed herein, the VH1 has an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to SEQ ID NO:14. In some embodiments of the bispecific antibodies

disclosed herein, the VL2 has an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to SEQ ID NO:15. In some embodiments of the bispecific antibodies disclosed herein, the VH2 has an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to SEQ ID NO:16, respectively.

[0082] In some embodiments of the bispecific antibodies disclosed herein, the VL1 and VH1 that specifically bind to human RANKL have the amino acid sequences of SEQ ID NO:13 (VL1) and SEQ ID NO:14 (VH1), respectively. In some embodiments of the bispecific antibodies disclosed herein, the VL2 and VH2 that specifically bind to human sclerostin have the amino acid sequences of SEQ ID NO:15 (VL2) and SEQ ID NO:16 (VH2), respectively.

6.2.2 Heterodimeric Fcs (*e.g.*, KIH)

[0083] In some embodiments, the bispecific antibodies provided herein comprise a mutation to promote heterodimerization of Fc regions. In some embodiments, a dimerized Fc region of a bispecific antibody provided herein is formed by Fc regions that contain amino acid mutations, substitutions, additions, or deletions to promote heterodimerization in which different polypeptides comprising different Fc regions can dimerize to yield a heterodimer configuration. In some embodiments, a bispecific antibody of the present disclosure comprises a first Fc sequence comprising a first CH3 region, and a second Fc sequence comprising a second CH3 region, wherein the sequences of the first and second CH3 regions are different and are such that the heterodimeric interaction between said first and second CH3 regions is stronger than each of the homodimeric interactions of said first and second CH3 regions.

[0084] Methods to promote heterodimerization of Fc regions include amino acid deletions, additions, or substitutions of the amino acid sequence of the Fc region, such as by including a set of "knobs-into-holes" deletions, additions, or substitutions or including amino acid deletions, additions, or substitutions to effect electrostatic steering of the Fc to favor attractive interactions among different polypeptide chains. Methods for promoting heterodimerization of complementary Fc polypeptides have been previously described in, for example, Ridgway *et al.*, 1996, *Protein Eng*, 9:617-621; Merchant *et al.*, 1998, *Nature Biotechnol*, 16:677-681; Moore *et al.*, 2011, *MAbs*, 3:546-557; Von Kreudenstein *et al.*, 2013, 5:646-654; Gunasekaran *et al.*, 2010, *J Biol Chem*, 285: 19637-19464; Leaver-Fay *et al.*, 2016, *Structure*, 24:641-651; Ha *et al.*, 2016, *Frontiers in Immunology*, 7: 1; Davis *et al.*, 2010, P*rotein Eng Des Sei*, 23: 195-202; W01996/027011; WO 1998/050431; W02006/028936; W02009/089004; WO2011/143545; WO2014/067011; WO2012/058768; WO2018/027025; US2014/0363426; US2015/0307628; US2018/0016354; US2015/0239991;

US2017/0058054; USPN5731168; USPN7183076; USPN9701759; USPN9605084; USPN9650446; USPN8216805; USPN8765412; and USPN8258268.

[0085] The modification promoting the association of a pair of Fc domains in a bispecific antibody includes the so-called "knobs-into-holes" modification, comprising a "knob" modification in one Fc domain and a "hole" modification in the other one. In some embodiments, the bispecific antibodies provided herein have complementary Fc polypeptides to form heterodimer of the "knobs-into-holes" configurations or "KIH" configuration. The "knobs-into-holes" technology is described *e.g.*, in US 5,731,168; US 7,695,936, US 8,216,805, US 8,765,412; Ridgway *et al.*, *Prot. Eng.* 9, 617-621 (1996) and Carter, *J Immunol. Meth.* 248, 7-15 (2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first Fc (the "Knob-Fc") and a corresponding cavity ("hole") in the interface of a second Fc (the "Hole-Fc"), such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine).

[0086] Accordingly, a "Knob-Fc region" and a "Hole-Fc region" are designed to form heterodimer pair. The Knob-Fc region refers to the Fc region in which an amino acid of the CH3 domain is replaced with an amino acid residue having a larger side chain volume, generating a protuberance within the CH3 domain positionable in a cavity within the CH3 domain of the Hole-Fc region, in which an amino acid residue of the CH3 domain is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain within which the protuberance within the CH3 domain of the first subunit is positionable. Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W). Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, *e.g.*, by site-specific mutagenesis, or by peptide synthesis.

[0087] In some embodiments, the threonine residue at position 366 of the Knob-Fc region is replaced with a tryptophan residue (T366W), and the tyrosine residue at position 407 of the Hole-Fc region is replaced with a valine residue (Y407V), and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A). Introduction of these two cysteine residues results in the formation of a

disulfide bridge between the two subunits of the Fc domain, thus further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)). Thus, in such configurations, a first Fc polypeptide comprises amino acid modifications to form the "knob" and a second Fc polypeptide comprises amino acid modifications to form the "hole" thus forming an Fc heterodimer comprising complementary Fc polypeptides. In some embodiments, the Knob-Fc region additionally has the serine residue at position 354 replaced with a cysteine residue (S354C), or the glutamic acid residue at position 356 replaced with a cysteine residue (E356C), and the Hole-Fc region additionally has the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C). In some embodiments, the Hole-Fc region additionally has the serine residue at position 354 replaced with a cysteine residue (S354C), or the glutamic acid residue at position 356 replaced with a cysteine residue (E356C), and the Knob-Fc region additionally has the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C). In some embodiments, the Knob-Fc region contains the amino acid substitutions S354C and T366W, and the Hole-Fc region contains the amino acid substitutions Y349C, T366S, L368A and Y407V. In some embodiments, the Knob-Fc region contains the amino acid substitutions Y349C and T366W, and the Hole-Fc region contains the amino acid substitutions S354C, T366S, L368A and Y407V. All amino acid residues are numbered according to the EU index.

[0088] In some embodiments, the bispecific antibodies provided herein have the "knobs-into-holes" or "KIH" structure (*e.g.*, FIG.1A). The "KIH" model promotes formation of heterodimers of the engineered bispecific antibody instead of heavy chain homodimers.

[0089] In some embodiments, complementary Fc polypeptides of an Fc heterodimer include a mutation to alter charge polarity across the Fc dimer interface such that co-expression of electrostatically matched Fc regions support favorable attractive interactions, thereby promoting desired Fc heterodimer formation; whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation (Guneskaran *et al.*, 2010, *J Biol Chem,* 285: 19637-19646). When co-expressed in a cell, association between the polypeptide chains is possible but the chains do not substantially self-associate due to charge repulsion.

[0090] Exemplary paired amino acid modifications of complementary Fc polypeptides of an Fc heterodimeric configuration are set forth below in the table below (EU numbering). (Brinkmann and Kontermann, *MAbs*. 2017 Feb-Mar; 9(2): 182–212.)

[0091] Table 4: Exemplary paired Fc modifications for heterodimeric Fc domains

First Fc polypeptide	**Second Fc polypeptide**
T366Y	Y407T

T366W	T366S/L368W/Y407V
T366W	T366S/L368A/Y407V
T366W/S354C	T366S/L368A/Y407V/Y349C
T366W/Y349C	T366S/L368A/Y407V/S354C
T366W/E356C	T366S/L368A/Y407V/Y349C
T366W/Y349C	T366S/L368A/Y407V/E356C
T350V/L351Y/F405A/Y407V	T350V/T366L/K392L/T394W
K360D/D399M/Y407A	E345R/Q347R/T366V/K409V
K409D/K392D	D399K/E356K
K360E/K409W	Q347R/D399V/F405T
L360E/K409W/Y349C	Q347R/D399V/F405T/S354C
S364H/F405A	Y349T/T394F
D221E/P228E/L368E	D221R/P228R/K409R
366K and/or 351K	D or E at 349, 368, and/or 355
F405L	K409R
Y349S/K307Y/T366M/K409V	E356G/E357D/S364Q/Y407A

[0092] The bispecific antibodies disclosed herein can comprise complementary Fc regions having the modifications described in Table 4 above. In some embodiments, the bispecific antibodies disclosed herein comprises a first CH region and a second CH region comprising paired Fc modification described in Table 4 above. In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have T366Y substitution and Y407T, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have T366W substitution and T366S/L368W/Y407V substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have T366W substitution and T366S/L368A/Y407V substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have T366W/S354C substitutions and T366S/L368A/Y407V/Y349C substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have T366W/E356C substitutions and T366S/L368A/Y407V/Y349C substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of

the bispecific antibodies disclosed herein have T350V/L351Y/F405A/Y407V substitutions and T350V/T366L/K392L/T394W substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have K360D/D399M/Y407A substitutions and E345R/Q347R/T366V/K409V substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have K409D/K392D substitutions and D399K/E356K substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have K360E/K409W substitutions and Q347R/D399V/F405T substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have L360E/K409W/Y349C substitutions and Q347R/D399V/F405T/S354C substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have S364H/F405A substitutions and Y349T/T394F substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have D221E/P228E/L368E substitutions and D221R/P228R/K409R substitutions, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have F405L substitution and K409R substitution, respectively, or *vice versa.* In some embodiments, the first CH region and the second CH region of the bispecific antibodies disclosed herein have Y349S/K307Y/T366M/K409V substitutions and E356G/E357D/S364Q/Y407A substitutions, respectively, or *vice versa.* In some embodiments, to further reduce formation of homodimers, one of the CH regions further has H435R and Y436F substitutions. In some embodiments, the second CH region further has H435R and Y436F substitutions.

[0093] In some embodiments, provided herein are bispecific antibodies comprising (i) a first light chain variable domain (VL1) and a first heavy chain variable domain (VH1), wherein the VL1/VH1 pair specifically binds to human RANKL and (ii) a second light chain variable domain (VL2) and a second heavy chain variable domain (VH2), wherein the VL2/VH2 pair specifically binds to human sclerostin. As shown in FIG.1A, to avoid mismatch, the KIH design includes an scFv on the second heavy chain. Accordingly, in some embodiments, the bispecific antibodies provided herein can have three peptide chains: (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1 and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant domain 1 (CH1), and a Knob-Fc region; and (3) a third

peptide chain (C3) comprising, from N-terminus to C-terminus, a linker, a single chain variable fragment (scFv) comprising VL2 and VH2, a linker, and a Hole-Fc region.

[0094] As a personal of ordinary skill in the art would understand, in the bispecific antibodies described above with the KIH configuration, the Knob-Fc and Hole-Fc can switch places. In other words, in the C2 and C3 pair of the bispecific antibodies that adopts the KIH design, C2 can includes a Knob-Fc region and the C3 can include a Hole-Fc region; alternatively, in some embodiments, the C2 can include a Hole-Fc region and the C3 can include a Knob-Fc region. Accordingly, in some embodiments, the bispecific antibodies provided herein can have three peptide chains: (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant domain 1 (CH1), and a Hole-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL2 and VH2, a linker, and a Knob-Fc region.

[0095] In an scFv, VL can be positioned at the N-terminal end of VH, or *vice versa.* In some embodiments of the bispecific antibodies disclosed herein, wherein C3 comprises an scFv having VL2 and VH2, the scFv can comprise, from N-terminus to C-terminus, VL2, a second linker, and VH2. In some embodiments, the scFv comprises, from N-terminus to C-terminus, VH2, a second linker, and VL2.

[0096] In some embodiments, provided herein are bispecific antibodies comprising a VL1/VH1 pair that specifically binds to human RANKL and a VL2/VH2 pair that specifically binds to human sclerostin. In some embodiments, the bispecific antibodies take the KIH configuration with an scFv on the second heavy chain. In some embodiments, the scFv comprises the VL2/VH2 pair that specifically binds to human sclerostin. In some embodiments, the scFv comprises the VL1/VH1 pair that specifically binds to human RANKL. As such, in some embodiments, the bispecific antibodies can have three peptide chains: (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant domain 1 (CH1), and a Knob-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL1 and VH1, a linker, and a Hole-Fc region. In some embodiments, the bispecific antibodies provided herein can have three peptide chains: (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant domain 1 (CH1), and a Hole-Fc region; and (3) a third peptide chain (C3)

comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL1 and VH1, a linker and a Knob-Fc region.

[0097] In some embodiments of the bispecific antibodies disclosed herein, wherein C3 comprises an scFv having VL1 and VH1, the scFv can comprise, from N-terminus to C-terminus, VL1, a second linker, and VH1. In some embodiments, the scFv comprises, from N-terminus to C-terminus, VH1, a second linker, and VL1.

[0098] The bispecific antibodies provided herein in KIH configuration comprise a CL region, a CH1 domain, and two Fc regions (Knob-Fc and Hole-Fc, each comprising a hinge, a CH2 domain and a CH3 domain). The amino acid sequences of the CH1, the CL region, and the Fc region of the bispecific antibodies disclosed herein can be derived from any appropriate source, *e.g.*, a constant region of an antibody such as an IgG1, IgG2, IgG3, or IgG4. Antibody heavy and light chain constant regions amino acid sequences are well known in the art, *e.g.*, those provided in the IMGT database (www.imgt.org) or at www.vbase2.org/vbstat.php., both of which are incorporated by reference herein.

[0099] In some embodiments, the constant domains and constant regions of the bispecific antibodies provided herein are derived from human IgG. In some embodiments, the constant domains and constant regions of the bispecific antibodies provided herein are derived from human IgG1. In some embodiments, the constant domains and constant regions of the bispecific antibodies provided herein are derived from human IgG2. In some embodiments, the constant domains and constant regions of the bispecific antibodies provided herein are derived from human IgG3. In some embodiments, the constant domains and constant regions of the bispecific antibodies provided herein are derived from human IgG4. In some embodiments, the amino acid sequences of the CH1, the CL region, and the Fc region (hinge, CH2 and CH3) of the bispecific antibodies disclosed herein can comprise one or more amino acid substitutions that differ from the wild type immunoglobulin. Such substitutions are known in the art (see, *e.g.*, US7704497, US7083784, US6821505, US 8323962, US6737056, and US7416727).

[00100] In some embodiments, the CH1 domain of the bispecific antibodies provided herein can be selected from the group consisting of a human IgG1 CH1 domain (SEQ ID NO:35), a human IgG2 CH1 domain (SEQ ID NO:36), a human IgG3 CH1 domain (SEQ ID NO:37), and a human IgG4 CH1 domain (SEQ ID NO:38). The CH1 domain can be a human IgG1 CH1 domain (SEQ ID NO:35) or a variant thereof having up to ten amino acids substitutions. The CH1 domain can be a human IgG2 CH1 domain (SEQ ID NO:36) or a variant thereof having up to ten amino acids substitutions. The CH1 domain can be a human IgG3 CH1 domain (SEQ ID NO:37) or a variant

thereof having up to ten amino acids substitutions. The CH1 domain can be a human IgG4 CH1 domain (SEQ ID NO:38) or a variant thereof having up to ten amino acids substitutions.

[00101] In some embodiments, the CL region of the bispecific antibodies provided herein can be kappa CL (Cκ; SEQ ID NO:19). In some embodiments, the CL region of the bispecific antibodies provided herein can be lambda CL (Cλ; SEQ ID NO:20).

[00102] In some embodiments, the Fc regions of the bispecific antibodies provided herein can be variants of the Fc region of human IgG2. In some embodiments, the Knob-Fc region is a human IgG2 Fc region variant having up to ten amino acids substitutions, including a T366W substitution; and the Hole-Fc region is a human IgG2 Fc region variant having up to ten amino acids substitutions, including a Y407V substitution. In some embodiments, the Hole-Fc region can further have T366S and L368A substitutions. In some embodiments, the Knob-Fc and Hole-Fc regions can further include S354C and Y349C substitutions, respectively. In some embodiments, the Knob-Fc and Hole-Fc regions can further include Y349C and S354C substitutions, respectively. All amino acid residues are numbered according to the EU Index.

[00103] In some embodiments, the Knob-Fc region and the Hole-Fc region can have the amino acid sequences of (1) SEQ ID NOs:29 and 32, respectively; (2) SEQ ID NOs:30 and 33, respectively; or (3) SEQ ID NOs:31 and 34, respectively. The Knob-Fc region and the Hole-Fc region can have the amino acid sequences of SEQ ID NOs:29 and 32, respectively. The Knob-Fc region and the Hole-Fc region can have the amino acid sequences of SEQ ID NOs:30 and 33, respectively. The Knob-Fc region and the Hole-Fc region can have the amino acid sequences of SEQ ID NOs:31 and 34, respectively.

[00104] Table 5: Exemplified Fc sequences in KIH models.

Exemplary Fc in KIH model	**Sequences**
Knob-Fc v8	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLWCL VKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:29)
Knob-Fc v11	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPCREEMTKNQVSLWCL VKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:30)
Knob-Fc v12	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVCTLPPSREEMTKNQVSLWCL

	VKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:31)
Hole-Fc v8	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLSCAV KGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:32)
Hole-Fc v11	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:33)
Hole-Fc v12	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPCREEMTKNQVSLSCAV KGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:34)

[00105] The bispecific antibodies provided herein KIH format also comprise a linker connecting the scFv and the Knob-Fc, specifically, the C-terminus of the scFv and the N-terminus of the Knob-Fc. The linker can be any suitable linker disclosed herein or otherwise known in the art. For example, the linker can be selected from those identified in Table 2. In some embodiments, the linker is a GS linker having the amino acid sequence of SEQ ID NO:41. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:42. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:43. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:44. In some embodiments, the linker has the amino acid sequence of SEQ ID NO:45.

[00106] In some embodiments of the scFv, the light chain variable domain (VL) and the heavy chain variable domain (VH) are connected by a second linker. The second linker can be any suitable linker disclosed herein or otherwise known in the art. For example, the second linker can be selected from those identified in Table 2. In some embodiments, the linker is a GS linker having the amino acid sequence of SEQ ID NO:41. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:42. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:43. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:44. In some embodiments, the linker has the amino acid sequence of SEQ ID NO:45.

[00107] In some embodiments of the bispecific antibodies provided herein, (i) the CL region is kappa CL (Cκ; SEQ ID NO:19) or lambda CL (Cλ; SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions; (ii) the CH1 domain is human IgG2 CH1 domain (SEQ ID NO:36) or a variant thereof having up to ten amino acids substitutions; and/or (iii) the Knob-Fc region and the Hole-Fc region have the amino acid sequences of (1) SEQ ID NOs:29 and 32, respectively, (2) SEQ

ID NOs:30 and 33, respectively, or (3) SEQ ID NOs:31 and 34, respectively, or a variant thereof having up to ten amino acids substitutions. In some embodiments, the CL region, CH1 domain, the Knob-Fc region and the Hole-Fc region have the amino acid sequences of SEQ ID NOs:19, 36, 29 and 32, respectively. In some embodiments, the CL region, CH1 domain, the Knob-Fc region and the Hole-Fc region have the amino acid sequences SEQ ID NOs:19, 36, 30 and 33, respectively. In some embodiments, the CL region, CH1 domain, the Knob-Fc region and the Hole-Fc region have the amino acid sequences SEQ ID NOs:19, 36, 31 and 34, respectively.

[00108] In some embodiments of the bispecific antibodies provided herein, the CL region is Cκ (SEQ ID NO:19), the CH1 domain is human IgG2 CH1 domain (SEQ ID NO:36), the Knob-Fc and Hole-Fc have the amino acid sequences of SEQ ID NOs:29 and 32, respectively. In some embodiments, the CL region is Cκ (SEQ ID NO:19), the CH1 domain is human IgG2 CH1 domain (SEQ ID NO:36), and the Knob-Fc region and the Hole-Fc region have the amino acid sequences of SEQ ID NOs:30 and 33, respectively. In some embodiments, the CL region is Cκ (SEQ ID NO:19), the CH1 domain is human IgG2 CH1 domain (SEQ ID NO:36), and the Knob-Fc region and the Hole-Fc region have the amino acid sequences of SEQ ID NOs:31 and 34, respectively.

[00109] Provided in Table 6A are diagrams of the three peptide chains of exemplary bispecific antibodies in KIH format.

[00110] Table 6A: Peptide chains of exemplary bispecific antibodies (KIH)

Ab	**C1**	**C2**	**C3**
1.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Knob-Fc (T366W)	VL2-(L)-VH2-(L)-IgG2 Hole-Fc (T366S, L368A, Y407V)
2.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Knob-Fc (S354C, T366W)	VL2-(L)-VH2-(L)-IgG2 Hole-Fc (Y349C, T366S, L368A, Y407V)
3.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Knob-Fc (Y349C, T366W)	VL2-(L)-VH2-(L)-IgG2 Hole-Fc (S354C, T366S, L368A, Y407V)
4.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Hole-Fc (T366S, L368A, Y407V)	VL2-(L)-VH2-(L)-IgG2 Knob-Fc (T366W)
5.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Hole-Fc (Y349C, T366S, L368A, Y407V)	VL2-(L)-VH2-(L)-IgG2 Knob-Fc (S354C, T366W)
6.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Hole-Fc (S354C, T366S, L368A, Y407V)	VL2-(L)-VH2-(L)-IgG2 Knob-Fc (Y349C, T366W)
7.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Knob-Fc (T366W)	VH2-(L)-VL2-(L)-IgG2 Hole-Fc (T366S, L368A, Y407V)
8.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Knob-Fc (S354C, T366W)	VH2-(L)-VL2-(L)-IgG2 Hole-Fc (Y349C, T366S, L368A, Y407V)
9.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Knob-Fc (Y349C, T366W)	VH2-(L)-VL2-(L)-IgG2 Hole-Fc (S354C, T366S, L368A, Y407V)

10.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Hole-Fc (T366S, L368A, Y407V)	VH2-(L)-VL2-(L)-IgG2 Knob-Fc (T366W)
11.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Hole-Fc (Y349C, T366S, L368A, Y407V)	VH2-(L)-VL2-(L)-IgG2 Knob-Fc (S354C, T366W)
12.	VL1-Cκ	VH1-IgG2 CH1-IgG2 Hole-Fc (S354C, T366S, L368A, Y407V)	VH2-(L)-VL2-(L)-IgG2 Knob-Fc (Y349C, T366W)
13.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Knob-Fc (T366W)	VL1-(L)-VH1-(L)-IgG2 Hole-Fc (T366S, L368A, Y407V)
14.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Knob-Fc (S354C, T366W)	VL1-(L)-VH1-(L)-IgG2 Hole-Fc (Y349C, T366S, L368A, Y407V)
15.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Knob-Fc (Y349C, T366W)	VL1-(L)-VH1-(L)-IgG2 Hole-Fc (S354C, T366S, L368A, Y407V)
16.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Hole-Fc (T366S, L368A, Y407V)	VL1-(L)-VH1-(L)-IgG2 Knob-Fc (T366W)
17.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Hole-Fc (Y349C, T366S, L368A, Y407V)	VL1-(L)-VH1-(L)-IgG2 Knob-Fc (S354C, T366W)
18.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Hole-Fc (S354C, T366S, L368A, Y407V)	VL1-(L)-VH1-(L)-IgG2 Knob-Fc (Y349C, T366W)
19.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Knob-Fc (T366W)	VH1-(L)-VL1-(L)-IgG2 Hole-Fc (T366S, L368A, Y407V)
20.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Knob-Fc (S354C, T366W)	VH1-(L)-VL1-(L)-IgG2 Hole-Fc (Y349C, T366S, L368A, Y407V)
21.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Knob-Fc (Y349C, T366W)	VH1-(L)-VL1-(L)-IgG2 Hole-Fc (S354C, T366S, L368A, Y407V)
22.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Hole-Fc (T366S, L368A, Y407V)	VH1-(L)-VL1-(L)-IgG2 Knob-Fc (T366W)
23.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Hole-Fc (Y349C, T366S, L368A, Y407V)	VH1-(L)-VL1-(L)-IgG2 Knob-Fc (S354C, T366W)
24.	VL2-Cκ	VH2-IgG2 CH1-IgG2 Hole-Fc (S354C, T366S, L368A, Y407V)	VH1-(L)-VL1-(L)-IgG2 Knob-Fc (Y349C, T366W)

Note: The VL1/VH1 pair specifically binds to RANKL (*e.g.*, SEQ ID NOs:13 and 14); the VL2/VH2 specifically binds to sclerostin (*e.g.*, SEQ ID NOs:15 and 16); Cκ refers to the kappa CL. (L): Linker

Table 6B: Sequences of Exemplary Bispecific Antibodies (KIH)

Ab	C1	C2	C3
DR-P7	EIVLTQSPGTLSLSPGE RATLSCRASQSVRGRY LAWYQQKPGQAPRLLI YGASSRATGIPDRFSGS GSGTDFTLTISRLEPED FAVFYCQQYGSSPRTF GQGTKVEIKRTVAAPS	EVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAM SWVRQAPGKGLEWVSG ITGSGGSTYYADSVKGR FTISRDNSKNTLYLQMN SLRAEDTAVYYCAKDP GTTVIMSWFDPWGQGT	EVQLVQSGAEVKKPGAS VKVSCKASGYTFTDYN MHWVRQAPGQGLEWM GEINPNSGGAGYNQKFK GRVTMTTDTSTSTAYME LRSLRSDDTAVYYCARL GYDDIYDDWYFDVWGQ

	VFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQ WKVDNALQSGNSQES VTEQDSKDSTYSLSSTL TLSKADYEKHKVYAC EVTHQGLSSPVTKSFN RGEC (SEQ ID NO:51)	LVTVSSASTKGPSVFPLA PCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYT CNVDHKPSNTKVDKTV ERKCCVECPPCPAPPVA GPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNA KTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYK CKVSNKGLPAPIEKTISK TKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKG FYPSDISVEWESNGQPE NNYKTTPPMLDSDGSFF LYSKLTVDKSRWQQGN VFSCSVMHEALHNHYT QKSLSLSPGK (SEQ ID NO:53)	GTTVTVSSGGGGSGGGG SGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCR ASQDISNYLNWYQQKP GKAPKLLIYYTSRLLSG VPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQGD TLPYTFGGGTKVEIKER KCCVECPPCPAPPVAGP SVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEV QFNWYVDGVEVHNAKT KPREEQFNSTFRVVSVL TVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTK GQPREPQVCTLPPSREE MTKNQVSLSCAVKGFY PSDISVEWESNGQPENN YKTTPPMLDSDGSFFLV SKLTVDKSRWQQGNVF SCSVMHEALHNHYTQK SLSLSPGK (SEQ ID NO:54)
DR-P8	EIVLTQSPGTLSLSPGE RATLSCRASQSVRGRY LAWYQQKPGQAPRLLI YGASSRATGIPDRFSGS GSGTDFTLTISRLEPED FAVFYCQQYGSSPRTF GQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQ WKVDNALQSGNSQES VTEQDSKDSTYSLSSTL TLSKADYEKHKVYAC EVTHQGLSSPVTKSFN RGEC (SEQ ID NO:51)	EVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAM SWVRQAPGKGLEWVSG ITGSGGSTYYADSVKGR FTISRDNSKNTLYLQMN SLRAEDTAVYYCAKDP GTTVIMSWFDPWGQGT LVTVSSASTKGPSVFPLA PCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYT CNVDHKPSNTKVDKTV ERKCCVECPPCPAPPVA GPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNA KTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYK CKVSNKGLPAPIEKTISK TKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKG FYPSDISVEWESNGQPE NNYKTTPPMLDSDGSFF LYSKLTVDKSRWQQGN VFSCSVMHEALHNHYT QKSLSLSPGK (SEQ ID NO:55)	DIQMTQSPSSLSASVGD RVTITCRASQDISNYLN WYQQKPGKAPKLLIYYT SRLLSGVPSRFSGSGSGT DFTLTISSLQPEDFATYY CQQGDTLPYTFGGGTKV EIKGGGGSGGGGSGGGG SGGGGSEVQLVQSGAEV KKPGASVKVSCKASGYT FTDYNMHWVRQAPGQG LEWMGEINPNSGGAGY NQKFKGRVTMTTDTSTS TAYMELRSLRSDDTAVY YCARLGYDDIYDDWYF DVWGQGTTVTVSSERK CCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVQ FNWYVDGVEVHNAKTK PREEQFNSTFRVVSVLT VVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTK GQPREPQVCTLPPSREE MTKNQVSLSCAVKGFY PSDISVEWESNGQPENN YKTTPPMLDSDGSFFLV SKLTVDKSRWQQGNVF SCSVMHEALHNHYTQK

			SLSLSPGK (SEQ ID NO:56)
DR-P15	EIVLTQSPGTLSLSPGE RATLSCRASQSVRGRY LAWYQQKPGQAPRLLI YGASSRATGIPDRFSGS GSGTDFTLTISRLEPED FAVFYCQQYGSSPRTF GQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQ WKVDNALQSGNSQES VTEQDSKDSTYSLSSTL TLSKADYEKHKVYAC EVTHQGLSSPVTKSFN RGEC (SEQ ID NO:51)	EVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAM SWVRQAPGKGLEWVSG ITGSGGSTYYADSVKGR FTISRDNSKNTLYLQMN SLRAEDTAVYYCAKDP GTTVIMSWFDPWGQGT LVTVSSASTKGPSVFPLA PCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYT CNVDHKPSNTKVDKTV ERKCCVECPPCPAPPVA GPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNA KTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYK CKVSNKGLPAPIEKTISK TKGQPREPQVYTLPPSR EEMTKNQVSLWCLVKG FYPSDISVEWESNGQPE NNYKTTPPMLDSDGSFF LYSKLTVDKSRWQQGN VFSCSVMHEALHNHYT QKSLSLSPGK (SEQ ID NO:57)	DIQMTQSPSSLSASVGD RVTITCRASQDISNYLN WYQQKPGKAPKLLIYYT SRLLSGVPSRFSGSGSGT DFTLTISSLQPEDFATYY CQQGDTLPYTFGGGTKV EIKGGGGSGGGGSGGGG SGGGGSEVQLVQSGAEV KKPGASVKVSCKASGYT FTDYNMHWVRQAPGQG LEWMGEINPNSGGAGY NQKFKGRVTMTTDTSTS TAYMELRSLRSDDTAVY YCARLGYDDIYDDWYF DVWGQGTTVTVSSERK CCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVQ FNWYVDGVEVHNAKTK PREEQFNSTFRVVSVLT VVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTK GQPREPQVYTLPPSREE MTKNQVSLSCAVKGFY PSDISVEWESNGQPENN YKTTPPMLDSDGSFFLV SKLTVDKSRWQQGNVF SCSVMHEALHNHYTQK SLSLSPGK (SEQ ID NO:58)
DR-P16	EIVLTQSPGTLSLSPGE RATLSCRASQSVRGRY LAWYQQKPGQAPRLLI YGASSRATGIPDRFSGS GSGTDFTLTISRLEPED FAVFYCQQYGSSPRTF GQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQ WKVDNALQSGNSQES VTEQDSKDSTYSLSSTL TLSKADYEKHKVYAC EVTHQGLSSPVTKSFN RGEC (SEQ ID NO:51)	EVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAM SWVRQAPGKGLEWVSG ITGSGGSTYYADSVKGR FTISRDNSKNTLYLQMN SLRAEDTAVYYCAKDP GTTVIMSWFDPWGQGT LVTVSSASTKGPSVFPLA PCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYT CNVDHKPSNTKVDKTV ERKCCVECPPCPAPPVA GPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNA KTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYK	DIQMTQSPSSLSASVGD RVTITCRASQDISNYLN WYQQKPGKAPKLLIYYT SRLLSGVPSRFSGSGSGT DFTLTISSLQPEDFATYY CQQGDTLPYTFGGGTKV EIKGGGGSGGGGSGGGG SGGGGSEVQLVQSGAEV KKPGASVKVSCKASGYT FTDYNMHWVRQAPGQG LEWMGEINPNSGGAGY NQKFKGRVTMTTDTSTS TAYMELRSLRSDDTAVY YCARLGYDDIYDDWYF DVWGQGTTVTVSSERK CCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVQ FNWYVDGVEVHNAKTK

		CKVSNKGLPAPIEKTISK TKGQPREPQVCTLPPSRE EMTKNQVSLWCLVKGF YPSDISVEWESNGQPEN NYKTTPPMLDSDGSFFL YSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:59)	PREEQFNSTFRVVSVLT VVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTK GQPREPQVYTLPPCREE MTKNQVSLSCAVKGFY PSDISVEWESNGQPENN YKTTPPMLDSDGSFFLV SKLTVDKSRWQQGNVF SCSVMHEALHNHYTQK SLSLSPGK (SEQ ID NO:60)

[00111] In some embodiments, provided herein are bispecific antibody that specifically bind to human RANKL and human sclerostin, wherein the bispecific antibody has a first peptide chain (C1), a second peptide chain (C2), and a third peptide chain (C3), wherein C1, C2 and C3 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of (1) SEQ ID NOs:51, 53, and 54, respectively; (2) SEQ ID NOs:51, 55, and 56, respectively; (3) SEQ ID NOs:51, 57, and 58, respectively; (4) SEQ ID NOs:51, 59, and 60, respectively. In some embodiments, C1, C2 and C3 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of SEQ ID NOs:51, 53, and 54, respectively. In some embodiments, C1, C2 and C3 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of SEQ ID NOs:51, 55, and 56, respectively. In some embodiments, C1, C2 and C3 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of SEQ ID NOs:51, 57, and 58, respectively. In some embodiments, C1, C2 and C3 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of SEQ ID NOs:51, 59, and 60, respectively.

6.2.3 IgG-scFv

[00112] In some embodiments, the bispecific antibodies provided herein have the "IgG-scFv" structure depicted in FIG.1B. In some embodiments, provided herein are bispecific antibodies comprising (i) a first light chain variable domain (VL1) and a first heavy chain variable domain (VH1), wherein the VL1/VH1 pair specifically binds to human RANKL and (ii) a second light chain variable domain (VL2) and a second heavy chain variable domain (VH2), wherein the VL2/VH2 pair specifically binds to human sclerostin. In some embodiments, the bispecific antibodies in IgG-scFv format comprises two peptide chains: (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1 and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant region (CH), a linker, a single chain

variable fragment (scFv) comprising VL2 and VH2. Alternatively, in some embodiments, the bispecific antibodies in IgG-scFv format comprises two peptide chains: (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2 and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant region (CH), a linker, a single chain variable fragment (scFv) comprising VL1 and VH1.

[00113] The bispecific antibodies provided herein in IgG-scFv format comprise a CL region and a CH region. The amino acid sequences of the CL region and the CH region of the bispecific antibodies disclosed herein can be derived from any appropriate source, *e.g.*, a constant region of an antibody such as an IgG1, IgG2, IgG3, or IgG4. In some embodiments, the constant regions of the bispecific antibodies provided herein are derived from human IgG. In some embodiments, the constant regions of the bispecific antibodies provided herein are derived from human IgG1. In some embodiments, the constant regions of the bispecific antibodies provided herein are derived from human IgG2. In some embodiments, the constant regions of the bispecific antibodies provided herein are derived from human IgG3. In some embodiments, the constant regions of the bispecific antibodies provided herein are derived from human IgG4. In some embodiments, the amino acid sequences of the CL region and the CH of the bispecific antibodies disclosed herein can comprise one or more amino acid substitutions that differ from the wildtype immunoglobulin. Such substitutions are known in the art (see, *e.g.*, US7704497, US7083784, US6821505, US 8323962, US6737056, and US7416727).

[00114] In some embodiments, the CL region of the bispecific antibodies provided herein can be kappa CL (Cκ; SEQ ID NO:19), or a variant thereof having up to ten amino acids substitutions. In some embodiments, the CL region of the bispecific antibodies provided herein can be lambda CL (Cλ; SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions.

[00115] In some embodiments, the CH region of the bispecific antibodies provided herein can be selected from the group consisting of a human IgG1 CH region (SEQ ID NO:21), a human IgG2 CH region (SEQ ID NO:22), a human IgG3 CH region (SEQ ID NO:23), and a human IgG4 CH region (SEQ ID NO:24), or a variant thereof having up to ten amino acid substitutions. In some embodiments, the CH region is human IgG2 CH region (SEQ ID NO:22) or a variant thereof having up to ten amino acid substitutions.

[00116] In some embodiments of the bispecific antibodies provided herein, (1) the CL region is Cκ (SEQ ID NO:19) or Cλ (SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions; or (2) the CH region is human IgG1 CH region (SEQ ID NO:21), IgG2 CH region

(SEQ ID NO:22), IgG3 CH region (SEQ ID NO:23), or IgG4 CH region (SEQ ID NO:24), or a variant thereof having up to ten amino acids substitutions; or both (1) and (2).

[00117] In some embodiments of the bispecific antibodies provided herein, the CL region is Cκ (SEQ ID NO:19), and the CH region is human IgG2 CH region (SEQ ID NO:22).

[00118] The bispecific antibodies provided herein in IgG-scFv format also comprise a linker connecting the heavy chain constant region (CH) and the scFv, specifically, the C-terminus of the CH and the N-terminus of the scFv. The linker can be any suitable linker disclosed herein or otherwise known in the art. For example, the linker can be selected from those identified in Table 2. In some embodiments, the linker is a GS linker having the amino acid sequence of SEQ ID NO:41. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:42. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:43. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:44. In some embodiments, the linker has the amino acid sequence of SEQ ID NO:45.

[00119] In some embodiments of the scFv that comprises a VL2 and VH2 pair, the VL2 and VH2 are connected by a second linker. In some embodiments of the scFv that comprises a VL1 and VH1 pair, the VL1 and VH1 are connected by a second linker. The second linker can be any suitable linker disclosed herein or otherwise known in the art. For example, the second linker can be selected from those identified in Table 2. In some embodiments, the linker is a GS linker having the amino acid sequence of SEQ ID NO:41. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:42. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:43. In some embodiments, the GS linker has the amino acid sequence of SEQ ID NO:44. In some embodiments, the linker has the amino acid sequence of SEQ ID NO:45.

[00120] Provided in Table 7A are diagrams of the two peptide chains of exemplary bispecific antibodies in IgG-scFv format.

[00121] Table 7A: Exemplary peptide chains of bispecific antibodies (IgG-scFv)

No.	C1	C2
25.	VL1-Cκ	VH1-IgG2 CH-(L)-VL2-(L)-VH2
26.	VL1-Cκ	VH1-IgG2 CH-(L)-VH2-(L)-VL2
27.	VL2-Cκ	VH2-IgG2 CH-(L)-VL1-(L)-VH1
28.	VL2-Cκ	VH2-IgG2 CH-(L)-VH1-(L)-VL1

Note: The VL1/VH1 pair specifically binds to RANKL (*e.g.*, SEQ ID NOs:13 and 14); the VL2/VH2 specifically binds to sclerostin (*e.g.*, SEQ ID NOs:15 and 16); Cκ refers to the kappa CL. (L): Linker

Table 7B: Sequences of Exemplary Bispecific Antibodies (IgG-scFv)

Ab	C1	C2
DR-P25	EIVLTQSPGTLSLSPGERATLSCRASQSVRGRYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVFYCQQYGSSPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:51)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGITGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPGTTVIMSWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLLSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDTLPYTFGGGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMHWVRQAPGQGLEWMGEINPNSGGAGYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARLGYDDIYDDWYFDVWGQGTTVTVSS (SEQ ID NO:61)
DR-P26	EIVLTQSPGTLSLSPGERATLSCRASQSVRGRYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVFYCQQYGSSPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:51)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGITGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPGTTVIMSWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMHWVRQAPGQGLEWMGEINPNSGGAGYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARLGYDDIYDDWYFDVWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLLSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDTLPYTFGGGTKVEIK (SEQ ID NO:62)
DR-P27	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLLSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDTLPYTFGGGTKVEIKRTVAAPSVFIFPPSDE	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMHWVRQAPGQGLEWMGEINPNSGGAGYNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARLGYDDIYDDWYFDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSV

	QLKSGTASVVCLLNNFY PREAKVQWKVDNALQS GNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTK SFNRGEC (SEQ ID NO:52)	FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVH QDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVE WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGG GGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGER ATLSCRASQSVRGRYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVFYCQ QYGSSPRTFGQGTKVEIKGGGGSGGGGSGGGGSG GGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSS YAMSWVRQAPGKGLEWVSGITGSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDP GTTVIMSWFDPWGQGTLVTVSS (SEQ ID NO:63)
DR-P 28	DIQMTQSPSSLSASVGD RVTITCRASQDISNYLN WYQQKPGKAPKLLIYYT SRLLSGVPSRFSGSGSGT DFTLTISSLQPEDFATYY CQQGDTLPYTFGGGTKV EIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFY PREAKVQWKVDNALQS GNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTK SFNRGEC (SEQ ID NO:52)	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNM HWVRQAPGQGLEWMGEINPNSGGAGYNQKFKGR VTMTTDTSTSTAYMELRSLRSDDTAVYYCARLGY DDIYDDWYFDVWGQGTTVTVSSASTKGPSVFPLAP CSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVH QDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVE WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGG GGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGG SLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGI TGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKDPGTTVIMSWFDPWGQGTLVTV SSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLS PGERATLSCRASQSVRGRYLAWYQQKPGQAPRLLI YGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV FYCQQYGSSPRTFGQGTKVEIK (SEQ ID NO:64)

[00122] In some embodiments, provided herein are bispecific antibody that specifically bind to human RANKL and human sclerostin, wherein the bispecific antibody has a first peptide chain (C1) and a second peptide chain (C2), wherein C1 and C2 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of (1) SEQ ID NOs:51 and 61 respectively; (2) SEQ ID NOs:51 and 62 respectively; (3) SEQ ID NOs:52 and 63 respectively; or SEQ ID NOs:52 and 64 respectively. In some embodiments, C1 and C2 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of SEQ ID NOs:51 and 61 respectively. In some embodiments, C1 and C2 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of SEQ ID NOs:51

and 62 respectively. In some embodiments, C1 and C2 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of SEQ ID NOs:52 and 63, respectively. In some embodiments, C1 and C2 have at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequences of SEQ ID NOs:52 and 64, respectively.

6.2.4 Variants

[00123] The present disclosure further contemplates additional variants and equivalents that are substantially homologous to the bispecific antibodies described herein. In some embodiments, it is desirable to improve the binding affinity of the antibody. In some embodiments, it is desirable to modulate biological properties of the antibody, including but not limited to, specificity, thermostability, expression level, effector function(s), glycosylation, immunogenicity, and/or solubility. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of an antibody, such as changing the number or position of glycosylation sites or altering membrane anchoring characteristics.

[00124] Antibodies comprising functional variants of the heavy chain, light chains, VL regions, VH regions, or one or more CDRs of the antibodies of the examples as also provided herein. A functional variant of a heavy chain, a light chain, VL, VH, or CDRs used in the context of an antibody still allows the antibody to retain at least a substantial proportion (at least about 90%, 95% or more) of functional features of the "reference" and/or "parent" antibody, including affinity and/or the specificity/selectivity, Fc inertness and PK parameters such as half-life, Tmax, Cmax. Such functional variants typically retain significant sequence identity to the parent antibody and/or have substantially similar length of heavy and light chains. Exemplary variants include those which differ from heavy and/or light chains, VH and/or VL, and/or CDR regions of the parent antibody sequences mainly by conservative substitutions, *e.g.*, 10, such as 9, 8, 7, 6, 5, 4, 3, 2 or 1 of the substitutions in the variant may be conservative amino acid residue replacements.

[00125] In some embodiments, a variant of a bispecific antibody disclosed herein can retain its ability to bind RANKL and sclerostin to a similar extent, the same extent, or to a higher extent, as the parent bispecific antibody. In some embodiments, the variant can be at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent antibody or antigen-binding fragment. In certain embodiments, a variant of a bispecific antibody disclosed herein comprises the amino acid sequence of the parent a bispecific antibody disclosed herein with one or more conservative amino acid substitution. Conservative amino acid substitutions are known in the art and

include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties.

[00126] In some embodiments, a variant of a bispecific antibody disclosed herein comprises the amino acid sequence of the parent antibody with one or more non-conservative amino acid substitutions. In some embodiments, a variant of a bispecific antibody disclosed herein comprises the amino acid sequence of the parent binding antibody with one or more non-conservative amino acid substitution, wherein the one or more non-conservative amino acid substitutions do not interfere with or inhibit one or more biological activities of the variant. In certain embodiments, the one or more conservative amino acid substitutions and/or the one or more non-conservative amino acid substitutions can enhance a biological activity of the variant, such that the biological activity of the functional variant is increased as compared to the parent antibody.

[00127] In some embodiments, the variant can have 1, 2, 3, 4, or 5 amino acid substitutions in the CDRs (*e.g.*, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2 and VL CDR3) of the binding moiety.

[00128] In some embodiments, the bispecific antibodies provided herein include modification in their Fc regions. In some embodiments, the modified antibodies (*e.g.*, modified Fc region) provide for altered effector functions that, in turn, affect the biological profile of the antibody. For example, in some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region reduces Fc receptor binding of the modified antibody as it circulates. In some embodiments, the constant region modifications reduce the immunogenicity of the antibody. In some embodiments, the constant region modifications increase the serum half-life of the antibody. In some embodiments, the constant region modifications reduce the serum half-life of the antibody. In some embodiments, the constant region modifications decrease or remove ADCC and/or complement dependent cytotoxicity (CDC) of the antibody. In some embodiments, specific amino acid substitutions in a human IgG2 Fc region with corresponding IgG1 or IgG4 residues reduce effector functions (*e.g.*, ADCC and CDC) in the modified antibody. In some embodiments, an antibody does not have one or more effector functions (*e.g.*, "effectorless" antibodies). In some embodiments, the antibody has no ADCC activity and/or no CDC activity. In some embodiments, the antibody does not bind an Fc receptor and/or complement factors. In some embodiments, the antibody has no effector function(s). In some embodiments, the constant region modifications increase or enhance ADCC and/or CDC of the antibody. In some embodiments, the constant region is modified to eliminate disulfide linkages or oligosaccharide moieties. In some embodiments, the constant region is modified

to add/substitute one or more amino acids to provide one or more cytotoxin, oligosaccharide, or carbohydrate attachment sites.

[00129] In some embodiments of the bispecific antibodies provided herein, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor. In some embodiments of the bispecific antibodies provided herein, the Fc domain comprises one or more amino acid substitution that reduces the effector function. The effector function can be complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, or any combination thereof. In some embodiments, the effector function is ADCC.

[00130] In some embodiments, variants can include addition of amino acid residues at the amino- and/or carboxyl-terminal end of the antibody. The length of additional amino acids residues can range from one residue to a hundred or more residues. In some embodiments, a variant comprises an N-terminal methionyl residue. In some embodiments, a variant is engineered to be detectable and may comprise a detectable label and/or protein (*e.g.*, a fluorescent tag or an enzyme).

[00131] The variant antibodies described herein can be generated using methods known in the art, including but not limited to, site-directed mutagenesis, alanine scanning mutagenesis, and PCR mutagenesis.

[00132] In some embodiments, bispecific antibodies disclosed herein can be chemically modified naturally or by intervention. In some embodiments, the bispecific antibodies are chemically modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, and/or linkage to a cellular ligand or other protein. Any of numerous chemical modifications can be carried out by known techniques. The bispecific antibodies provided herein can comprise one or more analogs of an amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art.

[00133] The bispecific antibodies of the present disclosure can be analyzed for their physical, chemical and/or biological properties by various methods known in the art. In some embodiments, a bispecific antibody provided herein is tested for its ability to bind human RANKL and sclerostin. Binding assays include, but are not limited to, BLI, SPR (*e.g.*, Biacore), ELISA, and FACS. In addition, antibodies can be evaluated for solubility, stability, thermostability, viscosity, expression levels, expression quality, and/or purification efficiency.

[00134] In some embodiments, bispecific antibodies disclosed herein can be conjugated to a detectable substance or molecule that allows the agent to be used for detection. A detectable substance can include, but is not limited to, enzymes, such as horseradish peroxidase, alkaline

phosphatase, beta-galactosidase, and acetylcholinesterase; prosthetic groups, such as biotin and flavine(s); fluorescent materials, such as, umbelliferone, fluorescein, fluorescein isothiocyanate (FITC), rhodamine, tetramethylrhodamine isothiocyanate (TRITC), dichlorotriazinylamine fluorescein, dansyl chloride, cyanine (Cy3), and phycoerythrin; bioluminescent materials, such as luciferase; radioactive materials; positron emitting metals; and magnetic metal ions positron emitting metals; and magnetic metal ions.

[00135] The anti-RANKL/sclerostin bispecific antibodies disclosed herein can be attached to a solid support. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride, or polypropylene. In some embodiments, an immobilized bispecific antibody is used in an immunoassay. In some embodiments, an immobilized bispecific antibody is used in purification.

6.3 Polynucleotides, vectors, and cells

[00136] Provided herein are polynucleotides encoding at least one light chain or one heavy chain of the anti-RANKL/sclerostin bispecific antibodies disclosed herein. In some embodiments, the polynucleotides provided herein encode one polypeptide, such as a light chain or a heavy chain of a bispecific antibody. In some embodiments, the polynucleotides provided herein encode more than one polypeptide. In some embodiments, the polynucleotides provided herein can encode, for example, the light chain and heavy chain of a bispecific antibody provided herein, respectively. Cistrons can be separated by, for example, an internal ribosomal entry site (IRES) or 2A element. An IRES, as understood in the art, refers to nucleotide sequences in an expression cassette which when transcribed into mRNA, can recruit ribosomes directly, without a previous scanning of untranslated region of mRNA by the ribosomes. A 2A element, as understood in the art, encoding self-cleaving short 2A peptides (about 20 amino acids) that provide a mechanism for subsequent separation of equimolarly produced polypeptides of interest. The family of self-cleaving 2A peptides has been described in the art (see for example, Kim, J.H. *et al.* (2011) *PLoS ONE* 6:el8556). The skilled artisan will appreciate that other art-recognized linkers may be suitable for use in the constructs of the disclosure (*e.g.*, encoded by the nucleic acids of the disclosure). The skilled artisan can likewise appreciate that other polycistronic constructs can be suitable for use as provided herein.

[00137] In some embodiments, provided herein are polynucleotides encoding the peptide chains C1, C2, C3, or any combination thereof, of the anti-RANKL/sclerostin bispecific antibodies disclosed herein having the KIH structure. In some embodiments, provided herein are polynucleotides encoding the LC, the HC, or both of the anti-RANKL/sclerostin bispecific antibodies disclosed herein having the IgG-ScFv structure.

[00138] In some embodiments, provided herein are polynucleotides encoding the C1, C2, C3, or any combination thereof, of the bispecific antibody designated as DR-P7. In some embodiments, provided herein are a plurality of polynucleotides that collectively encode the C1, C2, and C3 of the bispecific antibody designated as DR-P7. In some embodiments, provided herein are polynucleotides encoding the C1, C2, C3, or any combination thereof, of the bispecific antibody designated as DR-P8. In some embodiments, provided herein are a plurality of polynucleotides that collectively encode the C1, C2, and C3 of the bispecific antibody designated as DR-P8. In some embodiments, provided herein are polynucleotides encoding the C1, C2, C3, or any combination thereof, of the bispecific antibody designated as DR-P15. In some embodiments, provided herein are a plurality of polynucleotides that collectively encode the C1, C2, and C3 of the bispecific antibody designated as DR-P15. In some embodiments, provided herein are polynucleotides encoding the C1, C2, C3, or any combination thereof, of the bispecific antibody designated as DR-P16. In some embodiments, provided herein are a plurality of polynucleotides that collectively encode the C1, C2, and C3 of the bispecific antibody designated as DR-P16.

[00139] In some embodiments, provided herein are polynucleotides encoding the LC, the HC, or both, of the bispecific antibody designated as DR-P25. In some embodiments, provided herein are a first and a second polynucleotides that encode the LC and HC of the bispecific antibody designated as DR-P25, respectively. In some embodiments, provided herein are polynucleotides encoding the LC, the HC, or both, of the bispecific antibody designated as DR-P26. In some embodiments, provided herein are a first and a second polynucleotides that encode the LC and HC of the bispecific antibody designated as DR-P26, respectively. In some embodiments, provided herein are polynucleotides encoding the LC, the HC, or both, of the bispecific antibody designated as DR-P27. In some embodiments, provided herein are a first and a second polynucleotides that encode the LC and HC of the bispecific antibody designated as DR-P27, respectively. In some embodiments, provided herein are polynucleotides encoding the LC, the HC, or both, of the bispecific antibody designated as DR-P28. In some embodiments, provided herein are a first and a second polynucleotides that encode the LC and HC of the bispecific antibody designated as DR-P28, respectively.

[00140] The term "polynucleotide that encode a polypeptide" encompasses a polynucleotide which includes only coding sequences for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequences. The polynucleotides of the disclosure can be in the form of RNA or in the form of DNA. DNA can be cDNA, genomic DNA, or synthetic DNA, and can

be double-stranded or single-stranded. Single stranded DNA can be the coding strand or non-coding (anti-sense) strand. The polynucleotides of the disclosure can be mRNA.

[00141] The present disclosure also provides variants of the polynucleotides described herein, wherein the variants have a nucleotide sequence at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, or at least about 99% identical to a polynucleotide sequence encoding at least one polypeptide chain of an anti-RANKL/sclerostin bispecific antibody described herein. As used herein, the phrase "a polynucleotide having a nucleotide sequence at least about 95% identical to a polynucleotide sequence" means that the nucleotide sequence of the polynucleotide is identical to a reference sequence except that the polynucleotide sequence can include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence can be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence can be inserted into the reference sequence. These mutations of the reference sequence can occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[00142] The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some embodiments, a polynucleotide variant contains alterations which produce silent substitutions, additions, or deletions, but does not alter the properties or activities of the encoded polypeptide. In some embodiments, a polynucleotide variant comprises silent substitutions that results in no change to the amino acid sequence of the polypeptide (due to the degeneracy of the genetic code). Polynucleotide variants can be produced for a variety of reasons, for example, to optimize codon expression for a particular host (*e.g.*, change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*). In some embodiments, a polynucleotide variant comprises at least one silent mutation in a non-coding or a coding region of the sequence.

[00143] In some embodiments, a polynucleotide variant is produced to modulate or alter expression (or expression levels) of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to increase expression of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to decrease expression of the encoded polypeptide. In some embodiments, a polynucleotide variant has increased expression of the encoded polypeptide as compared to a parental

polynucleotide sequence. In some embodiments, a polynucleotide variant has decreased expression of the encoded polypeptide as compared to a parental polynucleotide sequence.

[00144] In some embodiments, a polynucleotide comprises the coding sequence for a polypeptide (*e.g.*, an antibody) fused in the same reading frame to a polynucleotide which aids in expression and secretion of a polypeptide from a host cell (*e.g.*, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide). The polypeptide can have the leader sequence cleaved by the host cell to form a "mature" form of the polypeptide.

[00145] In some embodiments, a polynucleotide comprises the coding sequence for a polypeptide (*e.g.*, an antibody) fused in the same reading frame to a marker or tag sequence. For example, in some embodiments, a marker sequence is a hexa-histidine tag (HIS-tag) that allows for efficient purification of the polypeptide fused to the marker. In some embodiments, a marker sequence is a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (*e.g.*, COS-7 cells) is used. In some embodiments, the marker sequence is a FLAG™ tag. In some embodiments, a marker can be used in conjunction with other markers or tags.

[00146] In some embodiments, a polynucleotide is isolated. In some embodiments, a polynucleotide is substantially pure.

[00147] In some embodiments, provided herein are also vectors comprising a polynucleotide disclosed herein. The term "vector," and its grammatical equivalents as used herein refer to a vehicle that is used to carry genetic material (*e.g.,* a polynucleotide sequence), which can be introduced into a host cell, where it can be replicated and/or expressed. Vectors applicable for use include, for example, expression vectors, plasmids, phage vectors, viral vectors, episomes and artificial chromosomes, which can include selection sequences or markers operable for stable integration into a host cell's chromosome. Additionally, the vectors can include one or more selectable marker genes and appropriate expression control sequences. Selectable marker genes that can be included, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like which are well known in the art. When two or more polynucleotides are to be co-expressed, both polynucleotides can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding polynucleotides can be operationally linked to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The introduction of polynucleotides into a host cell can be confirmed using methods well known in the art. It is understood by those skilled in the art

that the polynucleotides are expressed in a sufficient amount to produce a desired product, and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

[00148] In some embodiments, vectors provided herein can be expression vectors. In some embodiments, vectors provided herein comprise a polynucleotide encoding at least one polypeptide chain of the anti-RANKL/sclerostin bispecific antibodies described herein. In some embodiments, provided herein are recombinant expression vectors, which can be used to amplify and express a polynucleotide encoding at least one polypeptide chain of the anti-RANKL/sclerostin bispecific antibodies described herein. For example, a recombinant expression vector can be a replicable DNA construct that includes synthetic or cDNA-derived DNA fragments encoding at least one polypeptide chain of the anti-RANKL/sclerostin bispecific antibodies described herein, operatively linked to suitable transcriptional and/or translational regulatory elements derived from mammalian, microbial, viral or insect genes. In some embodiments, a viral vector is used. DNA regions are "operatively linked" when they are functionally related to each other. For example, a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. In some embodiments, structural elements intended for use in certain expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. In some embodiments, in situations where recombinant protein is expressed without a leader or transport sequence, a polypeptide can include an N-terminal methionine residue.

[00149] Examples of vectors are plasmid, autonomously replicating sequences, and transposable elements. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including pCR1, pBR322, pMB9 and their derivatives, and wider host range plasmids, such as M13 and other filamentous single-stranded DNA phages. Additional exemplary vectors include, without limitation, plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. Examples of categories of animal viruses useful as vectors include, without limitation, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40). Examples of expression vectors are pClneo vectors (Promega) for expression in mammalian cells; pLenti4/V5-DEST™, pLenti6/V5-DEST™, and pLenti6.2/V5-GW/lacZ (Invitrogen) for lentivirus-mediated gene transfer and expression in mammalian cells. Useful expression vectors for eukaryotic hosts include, for example,

vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. Exemplary transposon systems such as Sleeping Beauty and PiggyBac can be used, which can be stably integrated into the genome (*e.g.*, Ivics *et al.*, *Cell,* 91 (4): 501–510 (1997); Cadiñanos *et al.*, (2007) *Nucleic Acids Research.* 35 (12): e87).

[00150] In some embodiments, the vector is an episomal vector or a vector that is maintained extrachromosomally. As used herein, the term "episomal" refers to a vector that is able to replicate without integration into host's chromosomal DNA and without gradual loss from a dividing host cell also meaning that said vector replicates extrachromosomally or episomally. The vector is engineered to harbor the sequence coding for the origin of DNA replication or "ori" from a lymphotrophic herpes virus or a gamma herpesvirus, an adenovirus, SV40, a bovine papilloma virus, or a yeast, specifically a replication origin of a lymphotrophic herpes virus or a gamma herpesvirus corresponding to oriP of EBV. In some embodiments, the lymphotrophic herpes virus may be Epstein Barr virus (EBV), Kaposi's sarcoma herpes virus (KSHV), Herpes virus saimiri (HS), or Marek's disease virus (MDV). Epstein Barr virus (EBV) and Kaposi's sarcoma herpes virus (KSHV) are also examples of a gamma herpesvirus. Typically, the host cell comprises the viral replication transactivator protein that activates the replication.

[00151] "Expression control sequences," "control elements," or "regulatory sequences" present in an expression vector are those non-translated regions of the vector—origin of replication, selection cassettes, promoters, enhancers, translation initiation signals (Shine Dalgarno sequence or Kozak sequence) introns, a polyadenylation sequence, 5' and 3' untranslated regions—which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including ubiquitous promoters and inducible promoters can be used.

[00152] Illustrative ubiquitous expression control sequences that can be used in present disclosure include, but are not limited to, a cytomegalovirus (CMV) immediate early promoter, a viral simian virus 40 (SV40) promoter (*e.g.*, early or late), a Moloney murine leukemia virus (MoMLV) LTR promoter, a Rous sarcoma virus (RSV) LTR, a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and P11 promoters from vaccinia virus, an elongation factor 1-alpha (EF1a) promoter, early growth response 1 (EGR1), ferritin H (FerH), ferritin L (FerL), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), eukaryotic translation initiation factor 4A1 (EIF4A1), heat shock 70kDa protein 5 (HSPA5), heat shock protein 90kDa beta, member 1 (HSP90B1), heat shock protein 70kDa (HSP70), β-kinesin (β-KIN), the human ROSA 26 locus (Irions *et al.*, *Nature*

Biotechnology 25, 1477 - 1482 (2007)), a Ubiquitin C promoter (UBC), a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken β-actin (CAG) promoter, and a β-actin promoter.

[00153] Illustrative examples of inducible promoters/systems include, but are not limited to, steroid-inducible promoters such as promoters for genes encoding glucocorticoid or estrogen receptors (inducible by treatment with the corresponding hormone), metallothionine promoter (inducible by treatment with various heavy metals), MX-1 promoter (inducible by interferon), the "GeneSwitch" mifepristone-regulatable system (Sirin *et al.*, 2003, *Gene*, 323:67), the cumate inducible gene switch (WO 2002/088346), tetracycline-dependent regulatory systems, *etc.* The bispecific antibodies described herein can be produced by any method known in the art, including chemical synthesis and recombinant expression techniques. The practice of the invention employs, unless otherwise indicated, conventional techniques in molecular biology, microbiology, genetic analysis, recombinant DNA, organic chemistry, biochemistry, PCR, oligonucleotide synthesis and modification, nucleic acid hybridization, and related fields within the skill of the art. These techniques are described in the references cited herein and are fully explained in the literature. *See, e.g.*, Maniatis *et al.* (1982) MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press; Sambrook *et al.* (1989), MOLECULAR CLONING: A LABORATORY MANUAL, Second Edition, Cold Spring Harbor Laboratory Press; Sambrook *et al.* (2001) MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons (1987 and annual updates); CURRENT PROTOCOLS IN IMMUNOLOGY, John Wiley & Sons (1987 and annual updates) Gait (ed.) (1984) OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH, IRL Press; Eckstein (ed.) (1991) OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL APPROACH, IRL Press; Birren *et al.* (eds.) (1999) GENOME ANALYSIS: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press; Borrebaeck (ed.) (1995) ANTIBODY ENGINEERING, Second Edition, Oxford University Press; Lo (ed.) (2006) ANTIBODY ENGINEERING: METHODS AND PROTOCOLS (METHODS IN MOLECULAR BIOLOGY); Vol. 248, Humana Press, Inc; each of which is incorporated herein by reference in its entirety.

[00154] The present disclosure also provides cells comprising the polynucleotides disclosed herein that encode at least one polypeptide chain of the anti-RANKL/sclerostin bispecific antibodies described herein. In some embodiments, cells provided herein comprise a polynucleotide that encodes the C1, C2, and C3 of the anti-RANKL/sclerostin bispecific antibodies disclosed herein having the KIH structure. In some embodiments, cells provided herein comprise a plurality of the

polynucleotides that collectively encode the C1, C2, and C3 of the anti-RANKL/sclerostin bispecific antibodies disclosed herein having the KIH structure.

[00155] In some embodiments, cells provided herein comprise a polynucleotide that encodes both the LC and the HC of the anti-RANKL/sclerostin bispecific antibodies disclosed herein having the IgG-scFv structure. In some embodiments, cells provided herein comprise a first polynucleotide that encodes the LC and a second polynucleotide that encodes HC of the anti-RANKL/sclerostin bispecific antibodies disclosed herein having the IgG-scFv structure.

[00156] Cells comprising vectors disclosed herein are also contemplated. In some embodiments, provided herein are host cells comprising a vector comprising a polynucleotide disclosed herein. In some embodiments, host cells provided herein comprise a vector or multiple vectors that collectively comprise the polynucleotides encoding the polypeptide chains of the anti-RANKL/sclerostin bispecific antibodies described herein. In some embodiments, host cells provided herein produce the anti-RANKL/sclerostin bispecific antibodies described herein.

[00157] Examples of suitable mammalian host cell lines include, but are not limited to, COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants thereof. Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Expression of recombinant proteins in insect cell culture systems (*e.g.*, baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art.

6.4 Methods of production

[00158] Provided herein are also methods of producing the anti-RANKL/sclerostin bispecific antibodies disclosed herein. In some embodiments, the bispecific antibodies disclosed herein are comprised of more than one polypeptide chain, which can be produced separately or together. In some embodiments, methods provided herein produce at least one polypeptide chain of the bispecific antibodies disclosed herein. In some embodiments, methods provided herein produce all polypeptide chains of the bispecific antibodies disclosed herein.

[00159] The bispecific antibodies or polypeptides described herein can be produced and isolated using methods known in the art. Polyeptides can be synthesized, in whole or in part, using chemical methods (see, *e.g.*, Caruthers (1980). *Nucleic Acids Res. Symp. Ser.* 215; Horn (1980); and Banga, A.K., THERAPEUTIC PEPTIDES AND PROTEINS, FORMULATION, PROCESSING AND DELIVERY SYSTEMS (1995) Technomic Publishing Co., Lancaster, PA). Peptide synthesis can be performed using various solid phase techniques (see, *e.g.*, Roberge, *Science* 269:202 (1995); Merrifield, *Methods. Enzymol.* 289:3 (1997)) and automated synthesis may be achieved, *e.g.*, using the ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the manufacturer's instructions. Peptides can also be synthesized using combinatorial methodologies. Synthetic residues and polypeptides can be synthesized using a variety of procedures and methodologies known in the art (see, *e.g.*, ORGANIC SYNTHESES COLLECTIVE VOLUMES, Gilman, *et al.* (Eds) John Wiley & Sons, Inc., NY). Modified peptides can be produced by chemical modification methods (see, for example, Belousov, *Nucleic Acids Res.* 25:3440 (1997); Frenkel, *Free Radic. Biol. Med.* 19:373 (1995); and Blommers, *Biochemistry* 33:7886 (1994)). Peptide sequence variations, derivatives, substitutions and modifications can also be made using methods such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR based mutagenesis. Site-directed mutagenesis (Carter *et al.*, *Nucl. Acids Res.*, 13:4331 (1986); Zoller *et al.*, *Nucl. Acids Res.* 10:6487 (1987)), cassette mutagenesis (Wells *et al.*, *Gene* 34:315 (1985)), restriction selection mutagenesis (Wells *et al.*, *Philos. Trans. R. Soc. London SerA* 317:415 (1986)) and other techniques can be performed on cloned DNA to produce invention peptide sequences, variants, fusions and chimeras, and variations, derivatives, substitutions and modifications thereof.

[00160] A variety of host-expression vector systems can be utilized to recombinantly express the bispecific antibodies described herein or one or more of their polypeptide chains. Suitable host cells for expression include prokaryotes, yeast cells, insect cells, or higher eukaryotic cells under the control of appropriate promoters. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts, as well as methods of protein production, including antibody production are well-known in the art. Such host-expression systems represent vehicles by which the coding sequences of the bispecific antibodies described herein can be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate polynucleotide coding sequences, express the bispecific antibodies described herein *in situ*. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing coding sequences for the compounds described herein; yeast (*e.g.*,

Saccharomyces pichia) transformed with recombinant yeast expression vectors containing sequences encoding the compounds described herein; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baclovirus) containing the sequences encoding the compounds described herein; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing sequences encoding the molecules compounds described herein; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 293T, 3T3 cells, lymphotic cells (see U.S. Pat. No. 5,807,715), Per C.6 cells (human retinal cells developed by Crucell) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[00161] In bacterial systems, many expression vectors can be advantageously selected depending upon the use intended for the protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of the bispecific antibodies described herein, vectors which direct the expression of high levels of protein products that are readily purified can be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther *et al.* (1983), *EMBO J.* 2: 1791-1794); pIN vectors (Inouye *et al.* (1985), *Nucleic Acids Res.* 13:3101-3110; Van Heeke *et al.* (1989), *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors can also be used to express polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00162] Useful expression vectors for eukaryotic hosts include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. In mammalian host cells, a number of viral-based expression systems can be utilized. Examples of suitable mammalian host cell lines include, but are not limited to, COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants thereof. Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences, such as necessary

ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Expression of recombinant proteins in insect cell culture systems (*e.g.*, baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art. Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes.

[00163] In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products can be important for the function of the protein. For example, in certain embodiments, the antibodies described herein can be expressed as a single gene product (*e.g.*, as a single polypeptide chain, i.e., as a polyprotein precursor), requiring proteolytic cleavage by native or recombinant cellular mechanisms to form separate polypeptides of the bispecific antibodies described herein. The disclosure thus encompasses engineering a nucleic acid sequence to encode a polyprotein precursor molecule comprising the polypeptides of the bispecific antibodies described herein, which includes coding sequences capable of directing post translational cleavage of said polyprotein precursor. Post-translational cleavage of the polyprotein precursor results in the polypeptides of the bispecific antibodies described herein. The post translational cleavage of the precursor molecule comprising the polypeptides of the compounds described herein can occur *in vivo* (i.e., within the host cell by native or recombinant cell systems/mechanisms, *e.g.* furin cleavage at an appropriate site) or can occur *in vitro* (*e.g.* incubation of said polypeptide chain in a composition comprising proteases or peptidases of known activity and/or in a composition comprising conditions or reagents known to foster the desired proteolytic action). Purification and modification of recombinant proteins is well known in the art such that the design of the polyprotein precursor can include a number of embodiments readily appreciated by a skilled artisan. Any known proteases or peptidases known in the art can be used for the described modification of the precursor molecule.

[00164] Different host cells have characteristic and specific mechanisms for the post- translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 293T, 3T3, WI38, BT483, Hs578T, HTB2, BT20 and T47D, CRL7030 and Hs578Bst.

[00165] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express compounds described herein can be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the compounds described herein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the compounds described herein.

[00166] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler *et al.* (1977), *Cell* 11: 223-232), hypoxanthine-guanine phosphoribosyltransferase (Szybalska *et al.* (1992) *Bioessays* 14: 495- 500), and adenine phosphoribosyltransferase (Lowy *et al.* (1980), *Cell* 22: 817-823) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler *et al.* (1980) *PNAS* 77:3567-3570; O'Hare *et al.* (1981) *PNAS*, 78: 1527-1531); gpt, which confers resistance to mycophenolic acid (Mulligan *et al.* (1981) *PNAS*, 78: 2072-2076); neo, which confers resistance to the aminoglycoside G-418 (Tolstoshev (1993), *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan (1993), *Science* 260:926-932; and Morgan *et al.* (1993), *Ann. Rev. Biochem.* 62: 191-217) and hygro, which confers resistance to hygromycin (Santerre *et al.* (1984) *Gene* 30: 147-156). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY; Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli *et al.* (eds), 1994, CURRENT PROTOCOLS IN HUMAN GENETICS, John Wiley & Sons, NY.

[00167] The expression levels of bispecific antibodies described herein or their polypeptide chains can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3 (Academic Press, New York, 1987). When a marker in the vector system described herein is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the

number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of a protein of interest, production of the protein of interest will also increase (Crouse *et al.* (1983) *Mol. Cell. Biol.* 3:257-266).

[00168] The host cell can be co-transfected with more than one expression vectors, each encoding a polypeptide chain of a bispecific antibody described herein. The vectors can contain identical selectable markers which enable equal expression of all polypeptides. Alternatively, a single vector can be used which encodes two or more polypeptides. The coding sequences for the polypeptides of compounds described herein can comprise cDNA or genomic DNA.

[00169] Once a bispecific antibody described herein or polypeptide described herein has been recombinantly expressed, it can be purified by any method known in the art for purification of polypeptides, polyproteins or antibodies (*e.g.*, analogous to antibody purification schemes based on antigen selectivity) for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen (optionally after Protein A selection where the compound comprises an Fc domain (or portion thereof)), and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of polypeptides or antibodies.

[00170] Provided herein are methods of producing an anti-RANKL/sclerostin bispecific antibody described herein or a polypeptide chain of a bispecific antibody described herein, the method comprising obtaining a cell described herein and expressing the polynucleotide described herein in said cell. In some embodiments, methods provided herein comprising culturing the cells under conditions that allow expression of the bispecific antibody. In some embodiments, the method further comprises isolating and purifying a bispecific antibody or polypeptide chain described herein.

[00171] The bispecific antibodies described herein can be tested for binding to human RANKL and/or sclerostin by, for example, standard ELISA. Briefly, microtiter plates are coated with purified antigen, and then blocked with bovine serum albumin. Dilutions of antibody are added to each well and incubated. The plates are washed and incubated with secondary reagent (*e.g.*, for human antibodies, a goat-anti-human IgG Fc-specific polyclonal reagent) conjugated to horseradish peroxidase (HRP). After washing, the plates can be developed and analyzed by a spectrophotometer. Antibodies can be further tested by flow cytometry for binding to a cell line expressing human RANKL and/or sclerostin, but not to a control cell line that does not express the target antigen. Briefly, the binding of antibodies can be assessed by incubating RANKL and/or sclerostin expressing CHO cells with the bispecific antibody provided herein. The cells can be washed, and binding can be detected with an anti-human IgG Ab. Flow cytometric analyses can be performed using a FACS can flow cytometry (Becton Dickinson, San Jose, CA).

[00172] The anti-RANKL/sclerostin bispecific antibodies provided herein can be further tested for reactivity with the target antigen(s) by Western blotting, and other methods known in the art for analyzing binding affinity, cross-reactivity, and binding kinetics of various anti-RANKL/sclerostin bispecific antibodies described herein include, for example, biolayer interferometry (BLI) using, for example, Gator system (Probe Life) or the Octet-96 system (Sartorius AG), or BIACORE™ surface plasmon resonance (SPR) analysis using a BIACORE™ 2000 SPR instrument (Biacore AB, Uppsala, Sweden).

6.5 Pharmaceutical Compositions

[00173] Provided herein are also pharmaceutical compositions comprising the anti-RANKL/sclerostin bispecific antibodies disclosed herein. In some embodiments, the pharmaceutical composition comprises a therapeutically effective amount of the bispecific antibodies disclosed herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical compositions are useful in treating an inflammatory disease or an autoimmune disease.

[00174] The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" refers to a material that is suitable for drug administration to an individual along with an active agent without causing undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition. In some embodiments, the pharmaceutical compositions disclosed herein can comprise one or more of a buffer system, a preservative, a tonicity agent, a chelating agent, a stabilizer and/or a surfactant, as well as various combinations thereof. The use of preservatives, isotonic agents, chelating agents, stabilizers and surfactants in pharmaceutical compositions is well-known to the skilled person. Reference may be made to REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 19th edition, 1995.

[00175] In some embodiments, the pharmaceutical compositions provided herein comprise the anti-RANKL/sclerostin bispecific antibodies provided herein. The anti-RANKL/sclerostin bispecific antibodies can be present at various concentrations. In some embodiments, the pharmaceutical compositions provided herein comprise the anti-RANKL/sclerostin bispecific antibodies provided herein at 1-1000 mg/mL. In some embodiments, the pharmaceutical compositions comprise the anti-RANKL/sclerostin bispecific antibodies provided herein at 10-500 mg/mL, 10-400 mg/mL, 10-300 mg/mL, 10-200 mg/mL, 10-100 mg/mL, 20-100 mg/mL, or 50-100 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise the anti-RANKL/sclerostin bispecific antibodies provided herein at about 10 mg/mL, about 20 mg/mL, about 30 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 150 mg/mL, about 180 mg/mL, about 200 mg/mL, about 300

mg/mL, about 500 mg/mL, about 800 mg/mL, or about 1000 mg/mL. Dosages can be readily adjusted by those skilled in the art; for example, a decrease in purity may require an increase in dosage.

[00176] Pharmaceutically acceptable carriers that can be used in compositions provided herein include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In some embodiments, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active ingredient (*i.e.*, the anti-RANKL/sclerostin bispecific antibodies) can be coated in a material to protect the active ingredient from the action of acids and other natural conditions that can inactivate the active ingredient.

[00177] Provided herein are also pharmaceutical compositions or formulations that improve the stability of the anti-RANKL/sclerostin bispecific antibodies to allow for their long-term storage. In some embodiments, the pharmaceutical composition or formulation disclosed herein comprises: (a) the anti-RANKL/sclerostin bispecific antibodies disclosed herein; (b) a buffering agent; (c) a stabilizing agent; (d) a salt; (e) a bulking agent; and/or (f) a surfactant. In some embodiments, the pharmaceutical composition or formulation is stable for at least 1 month, at least 2 months, at least 3 months, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 5 years or more. In some embodiments, the pharmaceutical composition or formulation is stable when stored at 4°C, 25°C, or 40°C.

[00178] Buffering agents useful in the pharmaceutical compositions or formulations disclosed herein can be a weak acid or base used to maintain the acidity (pH) of a solution near a chosen value after the addition of another acid or base. Suitable buffering agents can maximize the stability of the pharmaceutical formulations by maintaining pH control of the formulation. Suitable buffering agents can also ensure physiological compatibility or optimize solubility. Rheology, viscosity and other properties can also depend on the pH of the formulation. Common buffering agents include, but are not limited to, histidine, citrate, succinate, acetate and phosphate. In some embodiments, a buffering agent comprises histidine (*e.g.*, L-histidine) with isotonicity agents and potentially pH adjustment with an acid or a base known in the art. In certain embodiments, the buffering agent is L- histidine. In certain embodiments, the pH of the formulation is maintained between about 2 and about 10, or between about 4 and about 8.

[00179] Stabilizing agents are added to a pharmaceutical product to stabilize that product. Such agents can stabilize proteins in different ways. Common stabilizing agents include, but are not

limited to, amino acids such as glycine, alanine, lysine, arginine, or threonine, carbohydrates such as glucose, sucrose, trehalose, rafftnose, or maltose, polyols such as glycerol, mannitol, sorbitol, cyclodextrins or destrans of any kind and molecular weight, or PEG. In some embodiments, the stabilizing agent is chosen to maximize the stability of antibodies in lyophilized preparations. In certain embodiments, the stabilizing agent is sucrose and/or arginine.

[00180] Bulking agents can be added to a pharmaceutical composition or formulation to add volume and mass to the product, thereby facilitating precise metering and handling thereof. Common bulking agents include, but are not limited to, lactose, sucrose, glucose, mannitol, sorbitol, calcium carbonate, or magnesium stearate.

[00181] Surfactants are amphipathic substances with lyophilic and lyophobic groups. A surfactant can be anionic, cationic, zwitterionic, or nonionic. Examples of nonionic surfactants include, but are not limited to, alkyl ethoxylate, nonylphenol ethoxylate, amine ethoxylate, polyethylene oxide, polypropylene oxide, fatty alcohols such as cetyl alcohol or oleyl alcohol, cocamide MEA, cocamide DEA, polysorbates, or dodecyl dimethylamine oxide. In some embodiments, the surfactant is polysorbate 20 or polysorbate 80.

[00182] In some embodiments, the pharmaceutical composition is an aqueous formulation. Such a formulation is typically a solution or a suspension, but can also include colloids, dispersions, emulsions, and multi-phase materials. The term "aqueous formulation" is defined as a formulation comprising at least 50% w/w water. Likewise, the term "aqueous solution" is defined as a solution comprising at least 50 % w/w water, and the term "aqueous suspension" is defined as a suspension comprising at least 50 % w/w water.

[00183] In some embodiments, the pharmaceutical compositions disclosed herein are freeze-dried, to which the physician or the patient adds solvents and/or diluents prior to use.

[00184] Pharmaceutical compositions disclosed herein can also include a pharmaceutically acceptable antioxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[00185] Examples of suitable aqueous and nonaqueous carriers that can be employed in the pharmaceutical compositions or formulations described herein include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof,

vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[00186] These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms can be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It can also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[00187] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. In some embodiments, provided herein is a pharmaceutical composition comprising the anti-RANKL/sclerostin bispecific antibodies or cells provided herein wherein the composition is suitable for local administration.

[00188] Pharmaceutical compositions or formulations typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, the compositions can include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[00189] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, some methods of preparation are vacuum drying and

freeze- drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00190] The amount of active ingredient which can be combined with a carrier material in the pharmaceutical compositions or formulations disclosed herein can vary. In some embodiments, the amount of active ingredient which can be combined with a carrier material is the amount that produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01 percent to about ninety-nine percent of active ingredient, from about 0.1 percent to about 70 percent, or from about 1 percent to about 30 percent of active ingredient in combination with a pharmaceutically acceptable carrier.

[00191] The pharmaceutical compositions disclosed herein can be prepared with carriers that protect the active ingredient against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and poly lactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See. e.g.*, SUSTAINED AND CONTROLLED RELEASE DRUG DELIVERY SYSTEMS, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[00192] Provided herein are also kits for preparation of pharmaceutical compositions having the anti-RANKL/sclerostin bispecific antibodies disclosed herein. In some embodiments, the kit comprises the anti-RANKL/sclerostin bispecific antibodies disclosed herein and a pharmaceutically acceptable carrier in one or more containers. In another embodiment, the kits can comprise the anti-RANKL/sclerostin bispecific antibodies disclosed herein for administration to a subject. In specific embodiments, the kits comprise instructions regarding the preparation and/or administration of the anti-RANKL/sclerostin bispecific antibodies.

6.6 Methods and Uses

[00193] The anti-RANKL/sclerostin bispecific antibodies provided herein can be used in medical treatment. Provided herein are also methods of preventing or treating osteoporosis in a subject in need thereof comprising administering to the subject in need thereof a therapeutically effective amount of the anti-RANKL/sclerostin bispecific antibodies disclosed herein. Provided herein are also methods of promoting bone growth in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the anti-RANKL/sclerostin bispecific antibodies disclosed herein. Provided herein are also methods of increasing bone mass comprising administering to the subject in a subject in need thereof a therapeutically effective amount of the anti-

RANKL/sclerostin bispecific antibodies disclosed herein. In some embodiments, the subject is a human. In some embodiments, the subject has primary osteoporosis. In some embodiments, the subject has secondary osteoporosis. In some embodiments, the subject is at risk of developing osteoporosis.

[00194] Provided herein are uses of the anti-RANKL/sclerostin bispecific antibodies disclosed herein as a medicament. Provided herein are also uses of the anti-RANKL/sclerostin bispecific antibodies disclosed herein in preventing or treating osteoporosis. Provided herein are also uses of the anti-RANKL/sclerostin bispecific antibodies disclosed herein in promoting bone growth or increasing bone mass. Provided herein are also uses of the anti-RANKL/sclerostin bispecific antibodies disclosed herein for the preparation of a medicament for preventing or treating osteoporosis. Provided herein are uses of the anti-RANKL/sclerostin bispecific antibodies disclosed herein for the preparation of a medicament for promoting bone growth or increasing bone mass.

[00195] The term “treat” and its grammatical equivalents as used herein in connection with a disease or a condition, or a subject having a disease or a condition refer to an action, intervention and/or measure that suppresses, eliminates, reduces, and/or ameliorates a symptom, the severity of the symptom, and/or the frequency of the symptom associated with the disease or disorder being treated. Treatment of osteoporosis can suppress, eliminate, reduce, or ameliorate the symptoms associated with osteoporosis. These symptoms primarily include bone loss, decreased bone density, and an increased risk of fractures, and the treatment aims to slow down the rate of bone loss, strengthen the bones, and reduce the likelihood of fractures.

[00196] The term "prevent" and its grammatical equivalents as used in connection with a disease or a condition, or a subject at risk of developing a disease or a condition, refer to actions taken to hinder, impede, or mitigate the occurrence, development, or progression of the disease or disorder. The goal of prevention is to reduce the risk, delay the onset, or completely avoid the manifestation of the disease or condition in susceptible individuals.

[00197] The term “administer” and its grammatical equivalents as used herein refer to the act of delivering, or causing to be delivered, a therapeutic or a pharmaceutical composition to the body of a subject by a method described herein or otherwise known in the art. The therapeutic can be a compound, a polypeptide, an antibody, a cell, or a population of cells. Administering a therapeutic or a pharmaceutical composition includes prescribing a therapeutic or a pharmaceutical composition to be delivered into the body of a subject. Exemplary forms of administration include oral dosage forms, such as tablets, capsules, syrups, suspensions; injectable dosage forms, such as intravenous (IV), intramuscular (IM), or intraperitoneal (IP); transdermal dosage forms, including creams, jellies,

powders, or patches; buccal dosage forms; inhalation powders, sprays, suspensions, and rectal suppositories.

[00198] The terms "effective amount," "therapeutically effective amount," and their grammatical equivalents as used herein refer to the administration of an agent to a subject, either alone or as a part of a pharmaceutical composition and either in a single dose or as part of a series of doses, in an amount that is capable of having any detectable, positive effect on any symptom, aspect, or characteristics of a disease, disorder or condition when administered to the subject. The therapeutically effective amount can be ascertained by measuring relevant physiological effects. The exact amount required varies from subject to subject, depending on the age, weight, and general condition of the subject, the severity of the condition being treated, the judgment of the clinician, and the like. An appropriate "effective amount" in any individual case can be determined by one of ordinary skill in the art using routine experimentation.

[00199] The term "subject" as used herein refers to any animal (*e.g.*, a mammal), including, but not limited to, humans, non-human primates, canines, felines, rodents, and the like, which is to be the recipient of a particular treatment. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats. A human subject who needs the treatment may be a human subject having, at risk for, or suspected of having a disease. A subject having a disease can be identified by routine medical examination, *e.g.*, a physical examination, a laboratory test, an organ functional test, a CT scan, or an ultrasound. A subject suspected of having any of such a disease can show one or more symptoms of the disease. A subject at risk for the disease can be a subject having one or more of the risk factors for that disease. A subject can be a human. A subject can have a particular disease or condition.

[00200] In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for treating or preventing osteoporosis. In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for treating or preventing primary osteoporosis. In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for treating postmenopausal osteoporosis (Type I). In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for treating senile osteoporosis (Type II). In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for treating idiopathic osteoporosis. In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for preventing postmenopausal osteoporosis (Type I). In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for preventing senile

osteoporosis (Type II). In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for preventing idiopathic osteoporosis.

[00201] In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for treating secondary osteoporosis. In some embodiments, the bispecific antibodies and pharmaceutical compositions provided herein can be used for preventing secondary osteoporosis. A person of ordinary skill in the art would understand that the secondary osteoporosis that can be treated or prevented with the bispecific antibodies and pharmaceutical compositions provided herein is not limited to any specific causes. For example, secondary osteoporosis with endocrine or metabolic causes, or associated with collagen/genetic disorders, medications, and/or nutritional deficiencies all can be treated with the bispecific antibodies and pharmaceutical compositions provided herein.

[00202] As such, subjects that can be treated with the bispecific antibodies and pharmaceutical compositions provided herein can have osteoporosis or are at risk of having osteoporosis. In some embodiments, the subject has been diagnosed with osteoporosis. In some embodiments, the subject has been diagnosed with Type I osteoporosis. In some embodiments, the subject has been diagnosed with Type II osteoporosis. In some embodiments, the subject has been diagnosed with secondary osteoporosis. In some embodiments, the subject is at risk of having osteoporosis. In some embodiments, the subject at risk of having osteoporosis is a postmenopausal woman. In some embodiments, the subject at risk of having osteoporosis is age 50 or older. In some embodiments, the subject at risk of having osteoporosis has a family history of osteoporosis or fractures. In some embodiments, the subject at risk of having osteoporosis has hormonal imbalances, who can have conditions like hypogonadism, hyperthyroidism, and/or adrenal disorders. In some embodiments, the subject at risk of having osteoporosis have certain medical conditions, such as rheumatoid arthritis, celiac disease, kidney disease, and hormone-related disorders. In some embodiments, the subject at risk of having osteoporosis has long-term use of medications like corticosteroids (prednisone), anticonvulsants, and some cancer treatments that weaken the bones. In some embodiments, the subject at risk of having osteoporosis has poor nutrition, low calcium and vitamin D intake, sedentary lifestyle, excessive alcohol consumption, smoking, and/or lack of weight-bearing exercise.

[00203] In some embodiments, methods provided herein prevent or treat osteoporosis in a subject. In some embodiments, methods provided herein prevent fractures in the subject. In some embodiments, methods provided herein improve bone density. In some embodiments, methods provided herein increase bone mass. In some embodiments, methods provided herein slow or halt bone loss. In some embodiments, methods provided herein prevent further deterioration of bone density. In some

embodiments, methods provided herein relieve symptoms associated with osteoporosis, including chronic pain. In some embodiments, methods provided herein enhance mobility and functionality of the subject. In some embodiments, methods provided herein minimize the risk of complications of osteoporosis, such as spinal deformities and loss of height.

[00204] Actual dosage levels of the active ingredients (*i.e.*, the anti-RANKL/sclerostin bispecific antibodies disclosed herein) in the pharmaceutical compositions described herein can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions described herein, the route of administration, the time of administration, the rate of excretion, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[00205] The anti-RANKL/sclerostin bispecific antibodies disclosed herein can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the anti-RANKL/sclerostin bispecific antibodies in the patient. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and until the patient shows partial or complete amelioration of symptoms of disease.

[00206] The anti-RANKL/sclerostin bispecific antibodies or pharmaceutical compositions provided herein can be administered to a subject by any methods known in the art, including, but not limited to, pleural administration, intravenous administration, subcutaneous administration, intranodal administration, intramuscular administration, intradermal administration, intrathecal administration, intrapleural administration, intraperitoneal administration, intracranial administration, spinal or other parenteral routes of administration, for example by injection or infusion, or direct administration to the thymus. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion. In some embodiments, subcutaneous administration is adopted. In some embodiments, intravenous administration is adopted. In some embodiments, oral administration is adopted. In one embodiment, the antibodies or

antigen-binding fragments provided herein can be delivered locally. In another embodiment, the antibodies or antigen-binding fragments provided herein can be administered systemically.

[00207] Anti-RANKL/sclerostin bispecific antibodies or pharmaceutical compositions provided herein can be administered with medical devices known in the art. For example, in some embodiments, a needleless hypodermic injection device can be used, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well- known implants and modules for use described herein include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicaments through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

[00208] In some embodiments, the anti-RANKL/sclerostin bispecific antibodies or pharmaceutical compositions provided herein can be administered with an additional therapy. The additional therapy can be administered prior to, concurrently with, or subsequent to administration of the bispecific antibodies or pharmaceutical compositions described herein. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. A person skilled in the art can readily determine appropriate regimens for administering a pharmaceutical composition described herein and an additional therapy in combination, including the timing and dosing of an additional agent to be used in a combination therapy, based on the needs of the subject being treated.

6.7 Exemplified embodiments

[00209] Embodiment 1: A bispecific antibody comprising (i) a first light chain variable domain (VL1) and a first heavy chain variable domain (VH1), wherein the VL1/VH1 pair specifically binds to human RANKL, and wherein the VL1 comprises VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively, and wherein the VH1 comprises VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5, and 6, respectively; and (ii) a second light chain variable domain (VL2) and a second heavy chain variable

domain (VH2), wherein the VL2/VH2 pair specifically binds to human sclerostin; and wherein the VL2 comprises VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:7, 8, and 9, respectively, and wherein the VH2 comprises VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:10, 11, and 12, respectively.

[00210] Embodiment 2: The bispecific antibody of Embodiment 1, wherein VL1, VH1, VL2, and VH2 have the amino acid sequences of SEQ ID NOs:13, 14, 15, and 16, respectively.

[00211] Embodiment 3: The bispecific antibody of Embodiment 1 or 2, comprising (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant domain 1 (CH1), and a Knob-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL2 and VH2, a linker, and a Hole-Fc region.

[00212] Embodiment 4: The bispecific antibody of Embodiment 1 or 2, comprising (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant domain 1 (CH1), and a Hole-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL2 and VH2, a linker, and a Knob-Fc region.

[00213] Embodiment 5: The bispecific antibody of Embodiment 3 or 4, wherein the scFv comprises, from N-terminus to C-terminus, VL2, a second linker, and VH2.

[00214] Embodiment 6: The bispecific antibody of Embodiment 3 or 4, wherein the scFv comprises, from N-terminus to C-terminus, VH2, a second linker, and VL2.

[00215] Embodiment 7: The bispecific antibody of Embodiment 1 or 2, comprising (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant domain 1 (CH1), and a Knob-Fc region; and (3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL1 and VH1, a linker, and a Hole-Fc region.

[00216] Embodiment 8: The bispecific antibody of Embodiment 1 or 2, comprising (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2, and a light chain constant region (CL); (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant domain 1 (CH1), and a Hole-Fc region; and (3) a third peptide chain (C3)

comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL1 and VH1, a linker and a Knob-Fc region.

[00217] Embodiment 9: The bispecific antibody of Embodiment 7 or 8, wherein the scFv comprises, from N-terminus to C-terminus, VL1, a second linker, and VH1.

[00218] Embodiment 10: The bispecific antibody of Embodiment 7 or 8, wherein the scFv comprises, from N-terminus to C-terminus, VH1, a second linker, and VL1.

[00219] Embodiment 11: The bispecific antibody of any one of Embodiments 3 to 10, wherein the Knob-Fc region is a human IgG2 Fc region variant having up to ten amino acids substitutions, including a T366W substitution; and the Hole-Fc region is a human IgG2 Fc region variant having up to ten amino acids substitutions, including T366S, L368A, Y407V substitutions.

[00220] Embodiment 12: The bispecific antibody of Embodiment 11, wherein the Knob-Fc region further comprises S354C substitution, and the Hole-Fc region further comprises Y349C substitution.

[00221] Embodiment 13: The bispecific antibody of Embodiment 11, wherein the Knob-Fc region further comprises Y349C substitution, and the Hole-Fc region further comprises S354C substitution.

[00222] Embodiment 14: The bispecific antibody of Embodiment 11, wherein (i) the CL region is kappa CL (Cκ; SEQ ID NO:19) or lambda CL (Cλ; SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions; (ii) the CH1 domain is human IgG2 CH1 domain (SEQ ID NO:36) or a variant thereof having up to ten amino acids substitutions; and/or (iii) the Knob-Fc region and the Hole-Fc region have the amino acid sequences of (1) SEQ ID NOs:29 and 32, respectively, (2) SEQ ID NOs:30 and 33, respectively, or (3) SEQ ID NOs:31 and 34, respectively, or a variant thereof having up to ten amino acids substitutions.

[00223] Embodiment 15: The bispecific antibody of Embodiment 14, wherein the CL region, CH1 domain, the Knob-Fc region and the Hole-Fc region have the amino acid sequences of (1) SEQ ID NOs:19, 36, 29 and 32, respectively, (2) SEQ ID NOs:19, 36, 30 and 33, respectively, or (3) SEQ ID NOs:19, 36, 31 and 34, respectively.

[00224] Embodiment 16: The bispecific antibody of Embodiment 3, wherein C1, C2, and C3 have amino acid sequences of (1) SEQ ID NOs:51, 53, and 54, respectively, (2) SEQ ID NOs:51, 55, and 56, respectively, (3) SEQ ID NOs:51, 57, and 58, respectively; or (4) SEQ ID NOs:51, 59 and 60, respectively.

[00225] Embodiment 17: A bispecific antibody of Embodiment 1 or 2, comprising: (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1 and a light chain constant region (CL); and (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1,

a heavy chain constant region (CH), a linker, and a single chain variable fragment (scFv) comprising VL2 and VH2.

[00226] Embodiment 18: The bispecific antibody of Embodiment 17, wherein the scFv comprises, from N-terminus to C-terminus, VL2, a second linker, and VH2.

[00227] Embodiment 19: The bispecific antibody of Embodiment 17, wherein the scFv comprises, from N-terminus to C-terminus, VH2, a second linker, and VL2.

[00228] Embodiment 20: A bispecific antibody of Embodiment 1 or 2, comprising: (1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2 and a light chain constant region (CL); and (2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant region (CH), a linker, and a single chain variable fragment (scFv) comprising VL1 and VH1.

[00229] Embodiment 21: The bispecific antibody of Embodiment 20, wherein the scFv comprises, from N-terminus to C-terminus, VL1, a second linker, and VH1.

[00230] Embodiment 22: The bispecific antibody of Embodiment 20, wherein the scFv comprises, from N-terminus to C-terminus, VH1, a second linker, and VL1.

[00231] Embodiment 23: The bispecific antibody of any one of Embodiments 17 to 22, wherein (1) the CL region is kappa CL (Cκ; SEQ ID NO:19) or lambda CL (Cλ; SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions; or (2) the CH region is human IgG1 CH region (SEQ ID NO:21), IgG2 CH region (SEQ ID NO:22), IgG3 CH region (SEQ ID NO:23), or IgG4 CH region (SEQ ID NO:24), or a variant thereof having up to ten amino acids substitutions; or both (1) and (2).

[00232] Embodiment 24: The bispecific antibody of Embodiment 23, wherein (1) the CL region is kappa CL (Cκ; SEQ ID NO:19); and (2) the CH region is human IgG2 CH region (SEQ ID NO:22).

[00233] Embodiment 25: The bispecific antibody of Embodiment 17, wherein the C1 and C2 have amino acid sequences of (1) SEQ ID NOs:51 and 61, respectively, (2) SEQ ID NOs:51 and 62, respectively.

[00234] Embodiment 26: The bispecific antibody of Embodiment 20, wherein the C1 and C2 have amino acid sequences of (1) SEQ ID NOs:52 and 63, respectively, (2) SEQ ID NOs:52 and 64, respectively.

[00235] Embodiment 27: A pharmaceutical composition comprising a therapeutically effective amount of the bispecific antibody of any one of Embodiments 1 to 26 and a pharmaceutically acceptable carrier.

[00236] Embodiment 28: A polynucleotide encoding a peptide chain of the bispecific antibody of any one of Embodiments 3 to 26.

[00237] Embodiment 29: The polynucleotide of Embodiment 28 encoding all peptide chains of the bispecific antibody.

[00238] Embodiment 30: A plurality of the polynucleotide of Embodiment 28 that collectively encode all peptide chains of the bispecific antibody.

[00239] Embodiment 31: A vector comprising the polynucleotide of Embodiment 28 or 29.

[00240] Embodiment 32: A cell comprising the polynucleotide or plurality of polynucleotides of any one of Embodiments 28 to 30, or the vector of Embodiment 31.

[00241] Embodiment 33: A method of making a bispecific antibody that specifically binds to human RANKL and human sclerostin comprising culturing the cell of Embodiment 32 under conditions that allow expression of the bispecific antibody.

[00242] Embodiment 34: A method of preventing or treating osteoporosis in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the bispecific antibody of any one of Embodiments 1 to 26.

[00243] Embodiment 35: The method of Embodiment 34, wherein the subject has primary osteoporosis.

[00244] Embodiment 36: The method of Embodiment 35, wherein the subject has postmenopausal osteoporosis (Type I).

[00245] Embodiment 37: The method of Embodiment 35, wherein the subject has senile osteoporosis (Type II).

[00246] Embodiment 38: The method of Embodiment 34, wherein the subject has secondary osteoporosis.

[00247] Embodiment 39: The method of Embodiment 38, wherein the osteoporosis is secondary to an endocrine or metabolic cause, a collagen or genetic disorder, a medication cause, or a nutritional cause.

[00248] Embodiment 40: The method of any one of Embodiments 34 to 39, wherein the subject is a human.

[00249] Embodiment 41: Use of the bispecific antibody of any one of Embodiments 1 to 26 as a medicament.

[00250] Embodiment 42: Use of the bispecific antibody of any one of Embodiments 1 to 26 in preventing or treating osteoporosis.

[00251] Embodiment 43: Use of the bispecific antibody of any one of Embodiments 1 to 26 for the preparation of a medicament for preventing or treating osteoporosis.

6.8 Experimental

[00252] The examples provided below are for purposes of illustration only, which are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

6.8.1 Example 1: Generation of asymmetric bispecific antibody (DRAb)

[00253] The bispecific IgG2 antibodies that specifically bound to both human RANKL and human sclerostin having the knobs-in-the-holes configuration were generated (FIGs.1A-1B; "DRAb"), including DR-P7, DR-P8, DR-P15, and DR-P16 (Table 6B). Point mutations were introduced in the CH3 domain of heavy chains. Specifically, in the "knobs" chain, S354C and T366W were introduced; in the "holes" chain, Y349C, T366S, L368A and Y407V were introduced. The "knobs" chain included the antibody binding fragment (Fab) targeting RANKL, whereas the "holes" chain included a single chain variable fragment (scFv) targeting sclerostin. As such, mispairing of light chain should be eliminated.

6.8.2 Example 2: Binding of DRAbs to its ligands

[00254] ELISA was used to examine the binding affinities of some candidate bispecific antibodies against human RANKL protein and sclerostin. Briefly, ELISA plate was coated overnight at 4 °C with his-tagged human RANKL at a concentration of 5 μg/mL. The coated-plate was washed with PBST buffer for 3 times and blocked with PBS in the presence of 1 % BSA for 2 hours at room temperature. After washing the blocked-plate with PBST for 3 times, the bispecific antibodies (50 μL) were added into the wells of his-tagged-human RANKL protein coated-ELISA plates at a series of concentrations (10 μg/mL being the highest concentration, 5-fold dilution, 7-point concentration) and incubated for 1 hour at room temperature. ELISA stripes were washed with PBST for 3 times and HRP-conjugated goat anti-human Fc specific antibody (Abcam, ab97225) at a dilution of 1:5000 in 1 % BSA in PBS was used to reveal the binding of the bispecific antibodies to the his-tagged RANKL. For the binding to sclerostin, his-tagged human sclerostin protein was coated at a concentration of 5 μg/mL and incubated overnight at 4 °C. ELISA was performed as described above. An exemplary result is provided in FIG.2. As shown, all three DRAbs: DR-P7, DR-P15, and DR-P16 specifically bound to human RANKL and human sclerostin. Denosumab and Romosozumab were used as reference antibodies.

6.8.3 Example 3: DRAbs effectively blocked osteoclast differentiation induced by RANKL

[00255] The blockade of DRAb on osteoclast differentiation was examined. RAW24.7, a murine macrophage precursor cell line, was activated with human RANKL for osteoclast differentiation. Briefly, 100 ng/mL human RANKL was incubated with RAW264.7 for 7 days. Human RANKL-containing medium was changed every 2-3 days. Thereafter, the cells were fixed with 4% paraformaldehyde for 5 mins at room temperature and washed once with PBS. Cells were stained according to the TRAcP kit procedure (Wako, Fujifilm), and photographed using a standard inverted microscope (Olympus, Japan). Mature osteoclasts were counted when more than three nuclei were present in the stained multinucleated cells. Denosumab was used as a reference antibody. An exemplary result is provided in FIG.3. As shown, bispecific antibodies disclosed herein, both DR-P7 and DR-P8 effectively inhibited osteoclast differentiation.

6.8.4 Example 4: DRAbs effectively inhibited induction of osteoclast marker gene expression by RANKL

[00256] The expression of marker genes for osteoclast differentiation was examined to confirm the inhibitory effect of DRAb. RAW24.7, a murine macrophage precursor cell line, was activated for osteoclast differentiation with human RANKL. Briefly, 100 ng/mL human RANKL was incubated with RAW264.7 for 7 days. Human RANKL-containing medium was changed every 2-3 days. Thereafter, RNA was collected with 500 μL RNAzol (MRC Inc, Ohio) and RNA was extracted according to manufacturer's protocol. 0.5 μg RNA was reverse transcribed to cDNA using PrimeScript RT Reagent Kit (Takara, Shiga). Quantitative PCR was performed using GoTaq® qPCR Master Mix and with the following cycling conditions: polymerase activation, 95 °C, 2 minutes; denaturation, 95 °C, 15 second; annealing and extension, 60 °C, 1 minute. 40 cycles of denaturation, annealing and extension were performed. Selected genes essential for osteoclast differentiation were examined. Denosumab was used as a reference antibody. An exemplary result is provided in FIG.4. As shown, both DRAbs DR-P7 and DR-P8 effectively inhibited expression of osteoclast marker genes.

6.8.5 Example 5: DRAbs effectively blocked sclerostin-mediated inhibition on WNT signaling in HEK293 cells.

[00257] HEK293 cells were used to assess the activities of DRAbs in blocking sclerostin-mediated inhibition of WNT signaling. The cells were plated at a density of 6,000 cells/cm^2 in 24-well culture plates, which were pre-coated with 50 μg/mL poly-D-lysine (Merck, A-003-E) for 1 hour. The cells were maintained in complete growth medium (Dulbecco's Modified Eagle Medium (Thermo Fisher

Scientific) containing 10% fetal bovine serum) at 37°C with 5% CO2. After 24 hours, the HEK293 cells were transfected with the pNL3.3 minP nanoluc TCE-LEF reporter plasmid (Promega) and a human WNT1-expressing plasmid (Genscript). The TCE-LEF reporter could monitor WNT signaling pathway activity. Twenty-four hours post-transfection, the cells were treated overnight with human sclerostin (Sino Biological) and an antibody at the designated concentration. The next day, a nanoluciferase assay (Promega) was performed to measure nanoluciferase levels in the culture medium, which indicated the activation level of the canonical WNT signaling pathway.

[00258] An exemplary result is provided in FIG.5. As shown, Romosozumab inhibited the sclerostin-mediated suppression of the WNT signaling pathway with an IC50 of 18.94 nM. DRAbs (DR-P7 and DR-P8) also inhibited sclerostin activity with IC50 values of 26.88 nM and 24.92 nM, respectively. These results demonstrate that DRAbs could effectively block sclerostin-mediated inhibition of the WNT signaling pathway in HEK293 cells.

6.8.6 Example 6: Bone formation in ovariectomized mice after antibody treatment

[00259] Transgenic mice expressing human RANKL (B6-hRANKL) were obtained from GemPharmatech Co., Ltd. Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at GemPharmatech. Female mice were housed in cages and maintained under a strict 12-h light:12-h dark cycle at 22°C with standard food pellets and free access to tap water. After a 1-week adaptation, 8-week-old female mice were randomly divided into following groups: Control group (ovary intact + vehicle (normal saline) i.v.), ovariectomy (OVX) group + IgG2 isotype (OVX + IgG), OVX + 5 mg/kg Denosumab group (OVX + Deno), OVX + 5 mg/kg Romosozumab group (OVX + Romo), OVX + 4 mg/kg DRAb group (OVX + DR-P7). On Day 0, ovariectomy was performed in a surgery room that had been exposed to ultraviolet radiation overnight. After anesthetization, the bilateral ovaries were exposed and removed to prepare the OVX animals; in the Control groups, the ovaries were exposed but left intact. Antibodies were administered intravenously weekly for 4 times starting from Day 0. Twenty-eight days after ovariectomy, mice were sacrificed, and their femurs were collected and fixed with 4% paraformaldehyde for 2 days. Samples were scanned using a Scanco micro-CT 40 system (Scanco Medical, Brütisellen, Switzerland) with settings of 70 kVP for power, 113 μA for current, and an integration time of 200 ms.

[00260] Gaussian filtering (sigma = 0.8, support = 1) was applied to reduce background noise. To analyze trabecular bone in the distal femur, the distal metaphysis was scanned at a nominal resolution of 10 μm/voxel. The analyzed region of interest began 0.03 mm proximal to the growth plate and extended 0.9 mm proximally. As shown in FIG.6, OVX treatment reduced trabecular number (Tb.N) and increased trabecular separation (Tb.Sp). Treatment with either Denosumab or Romosozumab

partially restored Tb.N and Tb.Sp. However, the group treated with DRAb showed a significantly greater increase in Tb.N and a more substantial reduction in Tb.Sp, demonstrating a synergistic effect in promoting bone regrowth by targeting both RANKL and sclerostin compared to targeting either one alone.

What is Claimed is:

1. A bispecific antibody comprising
(i) a first light chain variable domain (VL1) and a first heavy chain variable domain (VH1), wherein the VL1/VH1 pair specifically binds to human RANKL, and wherein the VL1 comprises VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively, and wherein the VH1 comprises VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5, and 6, respectively; and
(ii) a second light chain variable domain (VL2) and a second heavy chain variable domain (VH2), wherein the VL2/VH2 pair specifically binds to human sclerostin; and wherein the VL2 comprises VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:7, 8, and 9, respectively, and wherein the VH2 comprises VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:10, 11, and 12, respectively.

2. The bispecific antibody of claim 1, wherein VL1, VH1, VL2, and VH2 have the amino acid sequences of SEQ ID NOs:13, 14, 15, and 16, respectively.

3. The bispecific antibody of claim 1 or 2, comprising
(1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1, and a light chain constant region (CL);
(2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant domain 1 (CH1), and a Knob-Fc region; and
(3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL2 and VH2, a linker, and a Hole-Fc region.

4. The bispecific antibody of claim 1 or 2, comprising
(1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1, and a light chain constant region (CL);
(2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant domain 1 (CH1), and a Hole-Fc region; and
(3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL2 and VH2, a linker, and a Knob-Fc region.

5. The bispecific antibody of claim 3 or 4, wherein the scFv comprises, from N-terminus to C-terminus, VL2, a second linker, and VH2.

6. The bispecific antibody of claim 3 or 4, wherein the scFv comprises, from N-terminus to C-terminus, VH2, a second linker, and VL2.

7. The bispecific antibody of claim 1 or 2, comprising
(1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2, and a light chain constant region (CL);
(2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant domain 1 (CH1), and a Knob-Fc region; and
(3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL1 and VH1, a linker, and a Hole-Fc region.

8. The bispecific antibody of claim 1 or 2, comprising
(1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2, and a light chain constant region (CL);
(2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant domain 1 (CH1), and a Hole-Fc region; and
(3) a third peptide chain (C3) comprising, from N-terminus to C-terminus, a single chain variable fragment (scFv) comprising VL1 and VH1, a linker and a Knob-Fc region.

9. The bispecific antibody of claim 7 or 8, wherein the scFv comprises, from N-terminus to C-terminus, VL1, a second linker, and VH1.

10. The bispecific antibody of claim 7 or 8, wherein the scFv comprises, from N-terminus to C-terminus, VH1, a second linker, and VL1.

11. The bispecific antibody of any one of claims 3 to 10, wherein the Knob-Fc region is a human IgG2 Fc region variant having up to ten amino acids substitutions, including a T366W substitution; and the Hole-Fc region is a human IgG2 Fc region variant having up to ten amino acids substitutions, including T366S, L368A, Y407V substitutions.

12. The bispecific antibody of claim 11, wherein the Knob-Fc region further comprises S354C substitution, and the Hole-Fc region further comprises Y349C substitution.

13. The bispecific antibody of claim 11, wherein the Knob-Fc region further comprises Y349C substitution, and the Hole-Fc region further comprises S354C substitution.

14. The bispecific antibody of claim 11, wherein

(i) the CL region is kappa CL (Cκ; SEQ ID NO:19) or lambda CL (Cλ; SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions;
(ii) the CH1 domain is human IgG2 CH1 domain (SEQ ID NO:36) or a variant thereof having up to ten amino acids substitutions; and/or
(iii) the Knob-Fc region and the Hole-Fc region have the amino acid sequences of (1) SEQ ID NOs:29 and 32, respectively, (2) SEQ ID NOs:30 and 33, respectively, or (3) SEQ ID NOs:31 and 34, respectively, or a variant thereof having up to ten amino acids substitutions.

15. The bispecific antibody of claim 14, wherein the CL region, CH1 domain, the Knob-Fc region and the Hole-Fc region have the amino acid sequences of (1) SEQ ID NOs:19, 36, 29 and 32, respectively, (2) SEQ ID NOs:19, 36, 30 and 33, respectively, or (3) SEQ ID NOs:19, 36, 31 and 34, respectively.

16. The bispecific antibody of claim 3, wherein C1, C2, and C3 have amino acid sequences of (1) SEQ ID NOs:51, 53, and 54, respectively, (2) SEQ ID NOs:51, 55, and 56, respectively, (3) SEQ ID NOs:51, 57, and 58, respectively; or (4) SEQ ID NOs:51, 59 and 60, respectively.

17. A bispecific antibody of claim 1 or 2, comprising:
(1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL1 and a light chain constant region (CL); and
(2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH1, a heavy chain constant region (CH), a linker, and a single chain variable fragment (scFv) comprising VL2 and VH2.

18. The bispecific antibody of claim 17, wherein the scFv comprises, from N-terminus to C-terminus, VL2, a second linker, and VH2.

19. The bispecific antibody of claim 17, wherein the scFv comprises, from N-terminus to C-terminus, VH2, a second linker, and VL2.

20. A bispecific antibody of claim 1 or 2, comprising:
(1) a first peptide chain (C1) comprising, from N-terminus to C-terminus, VL2 and a light chain constant region (CL); and
(2) a second peptide chain (C2) comprising, from N-terminus to C-terminus, VH2, a heavy chain constant region (CH), a linker, and a single chain variable fragment (scFv) comprising VL1 and VH1.

21. The bispecific antibody of claim 20, wherein the scFv comprises, from N-terminus to C-terminus, VL1, a second linker, and VH1.

22. The bispecific antibody of claim 20, wherein the scFv comprises, from N-terminus to C-terminus, VH1, a second linker, and VL1.

23. The bispecific antibody of any one of claims 17 to 22, wherein
(1) the CL region is kappa CL (Cκ; SEQ ID NO:19) or lambda CL (Cλ; SEQ ID NO:20), or a variant thereof having up to ten amino acids substitutions; or
(2) the CH region is human IgG1 CH region (SEQ ID NO:21), IgG2 CH region (SEQ ID NO:22), IgG3 CH region (SEQ ID NO:23), or IgG4 CH region (SEQ ID NO:24), or a variant thereof having up to ten amino acids substitutions; or both (1) and (2).

24. The bispecific antibody of claim 23, wherein (1) the CL region is kappa CL (Cκ; SEQ ID NO:19); and (2) the CH region is human IgG2 CH region (SEQ ID NO:22).

25. The bispecific antibody of claim 17, wherein the C1 and C2 have amino acid sequences of (1) SEQ ID NOs:51 and 61, respectively, (2) SEQ ID NOs:51 and 62, respectively.

26. The bispecific antibody of claim 20, wherein the C1 and C2 have amino acid sequences of (1) SEQ ID NOs:52 and 63, respectively, (2) SEQ ID NOs:52 and 64, respectively.

27. A pharmaceutical composition comprising a therapeutically effective amount of the bispecific antibody of any one of claims 1 to 26 and a pharmaceutically acceptable carrier.

28. A polynucleotide encoding a peptide chain of the bispecific antibody of any one of claims 3 to 26.

29. The polynucleotide of claim 28 encoding all peptide chains of the bispecific antibody.

30. A plurality of the polynucleotide of claim 28 that collectively encode all peptide chains of the bispecific antibody.

31. A vector comprising the polynucleotide of claim 28 or 29.

32. A cell comprising the polynucleotide or plurality of polynucleotides of any one of claims 28 to 30, or the vector of claim 31.

33. A method of making a bispecific antibody that specifically binds to human RANKL and human sclerostin comprising culturing the cell of claim 32 under conditions that allow

expression of the bispecific antibody.

34. A method of preventing or treating osteoporosis in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the bispecific antibody of any one of claims 1 to 26.

35. The method of claim 34, wherein the subject has primary osteoporosis.

36. The method of claim 35, wherein the subject has postmenopausal osteoporosis (Type I).

37. The method of claim 35, wherein the subject has senile osteoporosis (Type II).

38. The method of claim 34, wherein the subject has secondary osteoporosis.

39. The method of claim 38, wherein the osteoporosis is secondary to an endocrine or metabolic cause, a collagen or genetic disorder, a medication cause, or a nutritional cause.

40. The method of any one of claims 34 to 39, wherein the subject is a human.

41. Use of the bispecific antibody of any one of claims 1 to 26 as a medicament.

42. Use of the bispecific antibody of any one of claims 1 to 26 in preventing or treating osteoporosis.

43. Use of the bispecific antibody of any one of claims 1 to 26 for the preparation of a medicament for preventing or treating osteoporosis.