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(57) Abstract: In certain aspects, the disclosure provides soluble heteromeric polypeptide complexes comprising an extracellular domain of a type I serine/threonine kinase receptor of the TGF-beta family and an extracellular domain of a type II serine/threonine kinase receptor of the TGF-beta family. In some embodiments, the disclosure provides soluble polypeptide complexes comprising an extracellular domain of a type II receptor selected from: ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII. In some embodiments, the disclosure provides soluble polypeptide complexes comprising an extracellular domain of a type I receptor selected from: ALK1, ALK2, ALK3, ALK4, ALK5, ALK.6, and ALK7. Optionally the soluble complex is a heterodimer. In certain aspects, such soluble polypeptide complexes may be used to regulate (promote or inhibit) growth of tissues or cells including, for example, muscle, bone, cartilage, fat, neural tissue, tumors, cancerous cells, and/or cells of hematopoietic lineages, including red blood cells. In certain aspects, such soluble polypeptide complexes are can be used to improve muscle formation, bone formation, hematopoiesis, metabolic parameters, and disorders associated with these tissues, cellular networks, and endocrine systems.

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TGF-BETA SUPERFAMILY TYPE I AND TYPE II RECEPTOR HETEROMULTIMERS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority from U.S. Provisional Application No. 62/404,563, filed October 5, 2016. The specification of the foregoing application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

The transforming growth factor-beta (TGF-beta) superfamily contains a variety of growth factors that share common sequence elements and structural motifs. These proteins are known to exert biological effects on a large variety of cell types in both vertebrates and invertebrates. Members of the superfamily perform important functions during embryonic development in pattern formation and tissue specification and can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. The family is divided into two general phylogenetic clades: the more recently evolved members of the superfamily, which includes TGF-betas, Activins, and nodal and the clade of more distantly related proteins of the superfamily, which includes a number of BMPs and GDFs. Hinck (2012) FEBS Letters 586:1860-1870. TGF-beta family members have diverse, often complementary biological effects. By manipulating the activity of a member of the TGF-beta family, it is often possible to cause significant physiological changes in an organism. For example, the Piedmontese and Belgian Blue cattle breeds carry a loss-of-function mutation in the GDF8 (also called myostatin) gene that causes a marked increase in muscle mass. Grobet *et al.* (1997) Nat Genet., 17(1):71-4. Furthermore, in humans, inactive alleles of GDF8 are associated with increased muscle mass and, reportedly, exceptional strength. Schuelke *et al.* (2004) N Engl J Med, 350:2682-8.

Changes in muscle, bone, fat, red blood cells, and other tissues may be achieved by enhancing or inhibiting signaling (*e.g.*, SMAD 1, 2, 3, 5, and/or 8) that is mediated by ligands of the TGF-beta family. Thus, there is a need for agents that regulate the activity of various ligands of the TGF-beta superfamily.

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Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

SUMMARY OF THE INVENTION

In one aspect, the present disclosure provides a recombinant heteromultimer comprising an ALK3-Fc fusion protein and an ActRIIB-Fc fusion protein,

(a) wherein ALK3-Fc fusion protein comprises:

(1) an ALK3 domain comprising an amino acid sequence that is at least 90% identical to amino acids 61-130 of SEQ ID NO: 22, and

(2) an Fc domain that is an IgG1 Fc domain, and wherein the IgG1 domain comprises a cysteine substitution at the position corresponding to S132 of SEQ ID NO: 3100 (S132C) and a tryptophan substitution at the position corresponding to T144 of SEQ ID NO: 3100 (T144W), wherein the amino acid substitutions alter the isoelectric point (pI) of the ALK3-Fc fusion protein; and

(b) wherein the ActRIIB-Fc fusion protein comprises:

(1) an ActRIIB domain comprising an amino acid sequence that is at least 90% identical to amino acids 29-109 of SEQ ID NO: 1, and

(2) an Fc domain that is an IgG1 Fc domain, and wherein the IgG1 Fc domain comprises one or more amino acid modifications that alter the pI of the ActRIIB-Fc fusion protein.

In another aspect, the present disclosure provides a pharmaceutical preparation comprising a heteromultimer of the invention and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure provides a recombinant nucleic acid comprising a coding sequence for an ALK3-Fc fusion protein as described herein.

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In another aspect, the present disclosure provides a recombinant nucleic acid comprising a coding sequence for an ActRIIB-Fc fusion protein as described herein.

In another aspect, the present disclosure provides a recombinant polynucleotide comprising a promoter sequence operably linked to one or more recombinant nucleic acids of the present invention.

In another aspect, the present disclosure provides a cell comprising a recombinant nucleic acid of the invention.

In another aspect, the present disclosure provides a method of making a heteromultimer comprising an ALK3-Fc fusion protein and a ActRIIB-Fc fusion protein comprising culturing a cell under conditions suitable for expression of an ALK3-Fc fusion protein and an ActRIIB-Fc fusion protein, wherein the cell comprises a recombinant nucleic acid of the invention.

In another aspect, the present disclosure provides a method of making a heteromultimer comprising an ALK3-Fc fusion protein and a ActRIIB-Fc fusion protein comprising culturing a cell under conditions suitable for expression of an ALK3-Fc fusion protein and an ActRIIB-Fc fusion protein, wherein the cell comprises a recombinant polynucleotide of the invention.

In another aspect, the present disclosure provides a method of making a heteromultimer comprising an ALK3-Fc fusion protein and a ActRIIB-Fc fusion protein comprising:

a) culturing a first cell under conditions suitable for expression of an ALK3-Fc fusion protein, wherein the first cell comprises a recombinant polynucleotide of the invention;

b) recovering the ALK3-Fc fusion protein so expressed;

c) culturing a second cell under conditions suitable for expression of a ActRIIB-Fc fusion protein, wherein the second cell comprises a recombinant polynucleotide of the invention;

d) recovering the ActRIIB-Fc fusion protein so expressed;

e) combining the recovered ALK3-Fc fusion protein and the recovered ActRIIB-Fc fusion protein under conditions suitable for type I:type II heteromultimer formation.

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In part, the disclosure provides heteromultimers comprising at least one TGF-beta superfamily type I serine/threonine kinase receptor polypeptide (*e.g.*, an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7 polypeptide), including fragments and variants thereof, and at least one TGF-beta superfamily type II serine/threonine kinase receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII), including fragments and variants thereof. In other aspects, the disclosure provides heteromultimers comprising at least two different TGF-beta superfamily type I serine/threonine kinase receptor polypeptide (*e.g.*, an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7 polypeptide), including fragments and variants thereof. In still other aspects, the disclosure provides heteromultimers comprising at least two different TGF-beta superfamily type II serine/threonine kinase receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII), including fragments and variants thereof. Optionally, heteromultimerics disclosed herein (*e.g.*, an ActRIIB:ALK4 heterodimer) have different ligand binding specificities/profiles compared to their corresponding homomultimers (*e.g.*, an ActRIIB homodimer and ALK4 homodimer). Novel properties, including novel ligand binding attributes, are exhibited by heteromultimeric polypeptide complexes comprising type I and type II receptor polypeptides of the TGF-beta superfamily, as shown by Examples herein.

Heteromultimeric structures include, for example, heterodimers, heterotrimers, and higher order complexes. See, e.g., Figures 1, 2, and 15. In some embodiments heteromultimers of the disclosure are heterodimers. Preferably, TGF-beta superfamily type I and type II receptor polypeptides as described herein comprise a ligand-binding domain of the receptor, for example, an extracellular domain of a TGF-beta superfamily type I or type II receptor. Accordingly, in certain aspects, protein complexes described herein comprise an extracellular domain of a type II TGF-beta superfamily receptor selected from: ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII, as well as truncations and variants thereof, and an extracellular domain of a type I TGF-beta superfamily receptor selected from: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7, as well as truncations and variants thereof. Preferably, TGF-beta superfamily type I and type II polypeptides as described herein, as well as protein complexes comprising the same, are soluble. In certain aspects, heteromultimers of the disclosure bind to one or more TGF-beta superfamily ligands (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-

β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, Müllerian-inhibiting substance (MIS), and Lefty). Optionally, protein complexes of the disclosure bind to one or more of these ligands with a K_D of greater than or equal to 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, or 10^{-12}. In general, heteromultimers of the disclosure antagonize (inhibit) one or more activities of at least one TGF-beta superfamily ligand, and such alterations in activity may be measured using various assays known in the art, including, for example, a cell-based assay as described herein. Preferably heteromultimers of the disclosure exhibit a serum half-life of at least 4, 6, 12, 24, 36, 48, or 72 hours in a mammal (*e.g.*, a mouse or a human). Optionally, heteromultimers of the disclosure may exhibit a serum half-life of at least 6, 8, 10, 12, 14, 20, 25, or 30 days in a mammal (*e.g.*, a mouse or a human).

T-beta superfamily type I receptor polypeptide and the amino acid sequence of a first member of an interaction pair and the second polypeptide comprises the amino acid sequence of a TGF-beta superfamily type II receptor polypeptide and the amino acid sequence of a second member of the interaction pair. In other aspects, heteromultimers described herein comprise a first polypeptide covalently or non-covalently associated with a second polypeptide wherein the first polypeptide comprises the amino acid sequence of a TGF-beta superfamily type I receptor polypeptide and the amino acid sequence of a first member of an interaction pair and the second polypeptide comprises the amino acid sequence of a different TGF-beta superfamily type I receptor polypeptide and the amino acid sequence of a second member of the interaction pair. In still other aspects, heteromultimers described herein comprise a first polypeptide covalently or non-covalently associated with a second polypeptide wherein the first polypeptide comprises the amino acid sequence of a TGF-beta superfamily type II receptor polypeptide and the amino acid sequence of a first member of an interaction pair and the second polypeptide comprises the amino acid sequence of a different TGF-beta superfamily type II receptor polypeptide and the amino acid sequence of a second member of the interaction pair. Optionally, the TGF-beta superfamily type I receptor polypeptide is connected directly to the first member of the interaction pair, or an intervening sequence, such as a linker, may be positioned between the amino acid sequence of the TGF-beta superfamily type I receptor polypeptide and the amino acid sequence of the first member of the interaction pair. Similarly, the TGF-beta superfamily type II receptor polypeptide may be connected directly to the second member of the interaction pair, or an intervening sequence, such as a linker, may be positioned between the amino acid sequence of the TGF-

beta superfamily type II receptor polypeptide and the amino acid sequence of the second member of the interaction pair. Linkers may correspond to the roughly 15 amino acid unstructured region at the C-terminal end of the extracellular domain of ActRIIB or ALK4 (the "tail"), or it may be an artificial sequence of between 5 and 15, 20, 30, 50, 100 or more amino acids that are relatively free of secondary structure. A linker may be rich in glycine and proline residues and may, for example, contain repeating sequences of threonine/serine and glycines. Examples of linkers include, but are not limited to, the sequences TGGG (SEQ ID NO: 62), TGGGG (SEQ ID NO: 60), SGGGG (SEQ ID NO: 61), GGGG (SEQ ID NO: 59), and GGG (SEQ ID NO: 58).

Interaction pairs described herein are designed to promote dimerization or form higher order multimers. In some embodiments, the interaction pair may be any two polypeptide sequences that interact to form a complex, particularly a heterodimeric complex although operative embodiments may also employ an interaction pair that forms a homodimeric sequence. The first and second members of the interaction pair may be an asymmetric pair, meaning that the members of the pair preferentially associate with each other rather than self-associate. Accordingly, first and second members of an asymmetric interaction pair may associate to form a heterodimeric complex. Alternatively, the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and thus may have the same or different amino acid sequences. Accordingly, first and second members of an unguided interaction pair may associate to form a homodimer complex or a heterodimeric complex. Optionally, the first member of the interaction action pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates covalently with the second member of the interaction pair. Optionally, the first member of the interaction action pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates non-covalently with the second member of the interaction pair. Optionally, the first member of the interaction pair (e.g., an asymmetrical or an unguided interaction pair) associates through both covalent and non-covalent mechanisms with the second member of the interaction pair.

In certain aspects, type I and/or type II polypeptides may be fusion proteins. For example, in some embodiments, an type I polypeptide may be a fusion protein comprising an type I polypeptide domain and one or more heterologous (non-type I) polypeptide domains (e.g., type I-Fc fusion proteins). Similarly, in some embodiments, an type II polypeptide may

be a fusion protein comprising an type II polypeptide domain and one or more heterologous (non-type II) polypeptide domains (type II-Fc fusion proteins).

In some embodiments, type I polypeptides are fusion proteins that comprise an Fc domain of an immunoglobulin. Similarly, in some embodiments, type II polypeptides are fusion proteins that comprise an Fc domain of an immunoglobulin. Traditional Fc fusion proteins and antibodies are examples of unguided interaction pairs, whereas a variety of engineered Fc domains have been designed as asymmetric interaction pairs [Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Therefore, a first member and/or a second member of an interaction pair described herein may comprise a constant domain of an immunoglobulin, including, for example, the Fc portion of an immunoglobulin. For example, a first member of an interaction pair may comprise an amino acid sequence that is derived from an Fc domain of an IgG (IgG1, IgG2, IgG3, or IgG4), IgA (IgA1 or IgA2), IgE, or IgM immunoglobulin. Such immunoglobulin domains may comprise one or more amino acid modifications (e.g., deletions, additions, and/or substitutions) that promote type I:type I, type II:type II, and/or type I:type II heteromultimer formation. Similarly, a second member of an interaction pair may comprise an amino acid sequence that is derived from an Fc domain of an IgG (IgG1, IgG2, IgG3, or IgG4), IgA (IgA1 or IgA2), IgE, or IgM. Such immunoglobulin domains may comprise one or more amino acid modifications (e.g., deletions, additions, and/or substitutions) that promote type I: type II heteromultimer formation. For example, the second member of an interaction pair may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to any one of SEQ ID NOs: 200-207, 3100, 3200, 3300, 3400 and 3500. In some embodiments, a first member and a second member of an interaction pair comprise Fc domains derived from the same immunoglobulin class and subtype. In other embodiments, a first member and a second member of an interaction pair comprise Fc domains derived from different immunoglobulin classes or subtypes.

In certain aspects, the disclosure relates to type I:type II heteromultimers comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein wherein the type I-Fc fusion protein comprises one or more amino acid modifications (e.g., amino acid substitution, cationization, deamination, carboxyl-terminal amino acid heterogeneity, phosphorylation, and glycosylation) that alter the isoelectric point (pI) of the type I-Fc fusion protein and/or the type II-Fc fusion protein comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments, the the one or more

amino acid modifications in the type I-Fc fusion protein confers increased difference in pIs between the type I-Fc fusion protein and the type II-Fc fusion protein. In other embodiments, the one or more amino acid modifications in the type II-Fc fusion protein confers increased difference in pIs between the type II-Fc fusion protein and the type I-Fc fusion protein. In still other embodiments the one or more amino acid modifications in the type I-Fc fusion protein confers increased difference in pIs between the type I-Fc fusion protein and the type II-Fc fusion protein, and the one or more amino acid modifications in the type II-Fc fusion protein confers increased difference in pIs between the type II-Fc fusion protein and the type I-Fc fusion protein. In some embodiments, the type I-Fc fusion protein comprises one or more amino acid modifications that alter pI by at least 0.1 (e.g., by at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.8. 0.9, 1.0, 1.3, 1.5, 1.7, 2.0, 2.3, 2.5, 2.7, 3.0, 3.3, 3.5, 3.7, or at least by 4.0). In some embodiments, the type II-Fc fusion protein comprises one or more amino acid modifications that alter pI by at least 0.1 (e.g., by at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.8. 0.9, 1.0, 1.3, 1.5, 1.7, 2.0, 2.3, 2.5, 2.7, 3.0, 3.3, 3.5, 3.7, or at least by 4.0). In some embodiments, the type I-Fc fusion protein comprises one or more amino acid modifications that alter pI by at least 0.1 (e.g., by at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.8. 0.9, 1.0, 1.3, 1.5, 1.7, 2.0, 2.3, 2.5, 2.7, 3.0, 3.3, 3.5, 3.7, or at least by 4.0) and the type II-Fc fusion protein comprises one or more amino acid modifications that alter pI by at least 0.1 (e.g., by at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.8. 0.9, 1.0, 1.3, 1.5, 1.7, 2.0, 2.3, 2.5, 2.7, 3.0, 3.3, 3.5, 3.7, or at least by 4.0). In some embodiments, the type I-Fc fusion protein and the type II-Fc fusion protein have at least a 0.7 difference in pI (e.g., at least 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3. 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, or at least 4.0 or more difference in pI).

In certain aspects, an type I:type II heteromultimer of the disclosure comprises an type I-Fc fusion protein comprising one or more amino acid modifications that increase the pI of the type I-Fc fusion protein; and an type II-Fc fusion protein comprising one or more amino acid modifications that decrease the pI of the type II-Fc fusion protein. For example, an type I-Fc fusion protein may be modified by substituting one or more neutral or negatively charged amino acids with one or more positively charged amino acids [e.g., an arginine (R), lysine (K), or histidine (H)]. Similarly, an type II-Fc fusion protein may be modified by substituting one or more neutral or positively charged amino acids with one or more negatively charged amino acids [e.g., aspartic acid (E) or glutamic acid (D)]. In some embodiments, the type I-Fc fusion protein Fc domain is an IgG1 Fc domain that comprises one or more amino acid modifications that alter the pI of the type I-Fc fusion protein. In

some embodiments, the type I-Fc fusion protein IgG1 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N162 of SEQ ID NO: 3100; b) an amino acid substitution at the position corresponding to D179 of SEQ ID NO: 3100; and c) an amino acid substitution at the position corresponding to N162 of SEQ ID NO: 3100 and an amino acid substitution at the position corresponding to D179 of SEQ ID NO: 3100. In some embodiments, the type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R, N162K, or N162H); b) an arginine, lysine, or histidine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R, D179K, or D179H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R, N162K. or N162H) and an arginine, lysine, or histidine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R, D179K. or D179H). In some embodiments, the type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R); b) an arginine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R); and c) an arginine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R) and an arginine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R). In some embodiments, the type I-Fc fusion protein Fc domain is an IgG2 Fc domain that comprises one or more amino acid modifications that alter the pI of the type I-Fc fusion protein. In some embodiments, the type I-Fc fusion protein IgG2 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the type I-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N160 of SEQ ID NO: 3200; b) an amino acid substitution at the position corresponding to D177 of SEQ ID NO: 3200; and c) an amino acid substitution at the position corresponding to N160 of SEQ ID NO: 3200 and an amino acid substitution at the position corresponding to D177 of SEQ ID NO: 3200. In some embodiments, the type I-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine,

or histidine substitution at the position corresponding to N160 of SEQ ID NO: 3200 (N160R, N160K, or N160H); b) an arginine, lysine, or histidine substitution at the position corresponding to D177 of SEQ ID NO: 3200 (D177R, D177K, or D177H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N160 of SEQ ID NO: 3200 (N160R, N160K, or N160H) and an arginine, lysine, or histidine substitution at the position corresponding to D177 of SEQ ID NO: 3200 (D177R, D177K. or D177H). In some embodiments, the type I-Fc fusion protein Fc domain is an IgG3 Fc domain that comprises one or more amino acid modifications that alter the pI of the type I-Fc fusion protein. In some embodiments, the type I-Fc fusion protein IgG3 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3300. In some embodiments, the type I-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to S169 of SEQ ID NO: 3300; b) an amino acid substitution at the position corresponding to D186 of SEQ ID NO: 3300; and c) an amino acid substitution at the position corresponding to S169 of SEQ ID NO: 3300 and an amino acid substitution at the position corresponding to D186 of SEQ ID NO: 3300. In some embodiments, the type I-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to S169 of SEQ ID NO: 3300 (S169R, S169K, or S169H); b) an arginine, lysine, or histidine substitution at the position corresponding to D186 of SEQ ID NO: 3300 (D186R, D186K, or D186H); and c) an arginine, lysine, or histidine substitution at the position corresponding to S169 of SEQ ID NO: 3300 (S169R, S169K, or S169H) and an arginine, lysine, or histidine substitution at the position corresponding to D186 of SEQ ID NO: 3300 (D186R, D186K, or D186H). In some embodiments, the type I-Fc fusion protein Fc domain is an IgG4 Fc domain that comprises one or more amino acid modifications that alter the pI of the type I-Fc fusion protein. In some embodiments, the type I-Fc fusion protein IgG4 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the type I-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N166 of SEQ ID NO: 3500; b) an amino acid substitution at the position corresponding to D183 of SEQ ID NO: 3500; and c) an amino acid substitution at the position corresponding to N166 of SEQ ID NO: 3500 and an amino acid substitution at the position corresponding to

D183 of SEQ ID NO: 3500. In some embodiments, the type I-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N166 of SEQ ID NO: 3500 (N166R, N166K, or N166H); b) an arginine, lysine, or histidine substitution at the position corresponding to D183 of SEQ ID NO: 3500 (D183R, D183K, or D183H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N166 of SEQ ID NO: 3500 (N166R, N166K, or N166H) and an arginine, lysine, or histidine substitution at the position corresponding to D183 of SEQ ID NO: 3500 (D183R, D183K. or D183H). In some embodiments, the type II-Fc fusion protein Fc domain is an IgG1 Fc domain that comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments, the type II-Fc fusion protein IgG1 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K138 of SEQ ID NO: 3100; b) an amino acid substitution at the position corresponding to K217 of SEQ ID NO: 3100; and c) an amino acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 and an amino acid substitution at the position corresponding to K217 of SEQ ID NO: 3100. In some embodiments, the type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E or K138D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217E or K217D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E or K138D) and an aspartic acid or glutamic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217E or K217D). In some embodiments, the type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) a glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E); b) an aspartic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217D); and c) a glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E) and an aspartic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217D). In some embodiments, the type II-Fc fusion protein Fc domain is an IgG2 Fc domain that comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments,

the type II-Fc fusion protein IgG2 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the type II-Fc fusion protein IgG2 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K136 of SEQ ID NO: 3200; b) an amino acid substitution at the position corresponding to K215 of SEQ ID NO: 3200; and c) an amino acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 and an amino acid substitution at the position corresponding to K215 of SEQ ID NO: 3200. In some embodiments, the type II-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 (K136E or K136D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K215 of SEQ ID NO: 3200 (K215E or K215D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 (K136E or K136D) and an aspartic acid or glutamic acid substitution at the position corresponding to K215 of SEQ ID NO: 3200 (K215E or K215D). In some embodiments, the type II-Fc fusion protein Fc domain is an IgG3 Fc domain that comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments, the type II-Fc fusion protein IgG3 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3300. In some embodiments, the type II-Fc fusion protein IgG3 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K145 of SEQ ID NO: 3300; b) an amino acid substitution at the position corresponding to K224 of SEQ ID NO: 3300; and c) an amino acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 and an amino acid substitution at the position corresponding to K224 of SEQ ID NO: 3300. In some embodiments, the modified type II-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 (K145E or K145D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K224 of SEQ ID NO: 3300 (K224E or K224D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 (K145E or K145D) and an aspartic acid or glutamic acid substitution at the position corresponding to K224 of SEQ ID NO: 3300 (K224E or K224D). In some embodiments, the type II-Fc fusion protein Fc domain is an

IgG4 Fc domain that comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments, the type II-Fc fusion protein IgG4 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the type II-Fc fusion protein IgG4 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K142 of SEQ ID NO: 3500; b) an amino acid substitution at the position corresponding to K221 of SEQ ID NO: 3500; and c) an amino acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 and an amino acid substitution at the position corresponding to K221 of SEQ ID NO: 3500. In some embodiments, the type II-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 (K142E or K142D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K221 of SEQ ID NO: 3500 (K221E or K221D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 (K142E or K142D) and an aspartic acid or glutamic acid substitution at the position corresponding to K221 of SEQ ID NO: 3500 (K221E or K221D).

In certain aspects, an type I:type II heteromultimer of the disclosure comprises an type II-Fc fusion protein comprising one or more amino acid modifications that increase the pI of the type II-Fc fusion protein; and an type I-Fc fusion protein comprising one or more amino acid modifications that decrease the pI of the type I-Fc fusion protein. For example, an type II-Fc fusion protein may be modified by substituting one or more neutral or negatively charged amino acids with one or more positively charged amino acids [e.g., an arginine (R), lysine (K), or histidine (H)]. Similarly, an type I-Fc fusion protein may be modified by substituting one or more neutral or positively charged amino acids with one or more negatively charged amino acids [e.g., aspartic acid (E) or glutamic acid (D)]. In some embodiments, the type II-Fc fusion protein Fc domain is an IgG1 Fc domain that comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments, the type II-Fc fusion protein IgG1 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N162 of SEQ ID NO: 3100; b) an amino acid substitution at the position corresponding to

D179 of SEQ ID NO: 3100; and c) an amino acid substitution at the position corresponding to N162 of SEQ ID NO: 3100 and an amino acid substitution at the position corresponding to D179 of SEQ ID NO: 3100. In some embodiments, the type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R, N162K, or N162H); b) an arginine, lysine, or histidine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R, D179K, or D179H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R, N162K. or N162H) and an arginine, lysine, or histidine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R, D179K. or D179H). In some embodiments, the type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R); b) an arginine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R); and c) an arginine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R) and an arginine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R). In some embodiments, the type II-Fc fusion protein Fc domain is an IgG2 Fc domain that comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments, the type II-Fc fusion protein IgG2 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the type II-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N160 of SEQ ID NO: 3200; b) an amino acid substitution at the position corresponding to D177 of SEQ ID NO: 3200; and c) an amino acid substitution at the position corresponding to N160 of SEQ ID NO: 3200 and an amino acid substitution at the position corresponding to D177 of SEQ ID NO: 3200. In some embodiments, the type II-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N160 of SEQ ID NO: 3200 (N160R, N160K, or N160H); b) an arginine, lysine, or histidine substitution at the position corresponding to D177 of SEQ ID NO: 3200 (D177R, D177K, or D177H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N160 of SEQ ID NO: 3200 (N160R, N160K, or N160H) and an arginine, lysine, or histidine substitution at the position corresponding to D177 of SEQ ID NO: 3200 (D177R, D177K. or D177H). In some

embodiments, the type II-Fc fusion protein Fc domain is an IgG3 Fc domain that comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments, the type II-Fc fusion protein IgG3 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3300. In some embodiments, the type II-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to S169 of SEQ ID NO: 3300; b) an amino acid substitution at the position corresponding to D186 of SEQ ID NO: 3300; and c) an amino acid substitution at the position corresponding to S169 of SEQ ID NO: 3300 and an amino acid substitution at the position corresponding to D186 of SEQ ID NO: 3300. In some embodiments, the type II-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to S169 of SEQ ID NO: 3300 (S169R, S169K, or S169H); b) an arginine, lysine, or histidine substitution at the position corresponding to D186 of SEQ ID NO: 3300 (D186R, D186K, or D186H); and c) an arginine, lysine, or histidine substitution at the position corresponding to S169 of SEQ ID NO: 3300 (S169R, S169K, or S169H) and an arginine, lysine, or histidine substitution at the position corresponding to D186 of SEQ ID NO: 3300 (D186R, D186K, or D186H). In some embodiments, the type II-Fc fusion protein Fc domain is an IgG4 Fc domain that comprises one or more amino acid modifications that alter the pI of the type II-Fc fusion protein. In some embodiments, the type II-Fc fusion protein IgG4 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the type II-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N166 of SEQ ID NO: 3500; b) an amino acid substitution at the position corresponding to D183 of SEQ ID NO: 3500; and c) an amino acid substitution at the position corresponding to N166 of SEQ ID NO: 3500 and an amino acid substitution at the position corresponding to D183 of SEQ ID NO: 3500. In some embodiments, the type II-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N166 of SEQ ID NO: 3500 (N166R, N166K, or N166H); b) an arginine, lysine, or histidine substitution at the position corresponding to D183 of SEQ ID NO: 3500 (D183R, D183K, or D183H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N166 of SEQ ID NO: 3500

(N166R, N166K, or N166H) and an arginine, lysine, or histidine substitution at the position corresponding to D183 of SEQ ID NO: 3500 (D183R, D183K. or D183H). In some embodiments, the type I-Fc fusion protein Fc domain is an IgG1 Fc domain that comprises one or more amino acid modifications that alter the pI of the type I-Fc fusion protein. In some embodiments, the type I-Fc fusion protein IgG1 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K138 of SEQ ID NO: 3100; b) an amino acid substitution at the position corresponding to K217 of SEQ ID NO: 3100; and c) an amino acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 and an amino acid substitution at the position corresponding to K217 of SEQ ID NO: 3100. In some embodiments, the type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E or K138D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217E or K217D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E or K138D) and an aspartic acid or glutamic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217E or K217D). In some embodiments, the type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) a glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E); b) an aspartic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217D); and c) a glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E) and an aspartic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217D). In some embodiments, the type I-Fc fusion protein Fc domain is an IgG2 Fc domain that comprises one or more amino acid modifications that alter the pI of the type I-Fc fusion protein. In some embodiments, the type I-Fc fusion protein IgG2 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the type I-Fc fusion protein IgG2 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K136 of SEQ ID NO: 3200; b) an amino acid substitution at the position corresponding to K215 of SEQ ID NO: 3200; and c)

an amino acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 and an amino acid substitution at the position corresponding to K215 of SEQ ID NO: 3200. In some embodiments, the type I-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 (K136E or K136D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K215 of SEQ ID NO: 3200 (K215E or K215D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 (K136E or K136D) and an aspartic acid or glutamic acid substitution at the position corresponding to K215 of SEQ ID NO: 3200 (K215E or K215D). In some embodiments, the type I-Fc fusion protein Fc domain is an IgG3 Fc domain that comprises one or more amino acid modifications that alter the pI of the type I-Fc fusion protein. In some embodiments, the type I-Fc fusion protein IgG3 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3300. In some embodiments, the type I-Fc fusion protein IgG3 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K145 of SEQ ID NO: 3300; b) an amino acid substitution at the position corresponding to K224 of SEQ ID NO: 3300; and c) an amino acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 and an amino acid substitution at the position corresponding to K224 of SEQ ID NO: 3300. In some embodiments, the modified type I-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 (K145E or K145D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K224 of SEQ ID NO: 3300 (K224E or K224D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 (K145E or K145D) and an aspartic acid or glutamic acid substitution at the position corresponding to K224 of SEQ ID NO: 3300 (K224E or K224D). In some embodiments, the type I-Fc fusion protein Fc domain is an IgG4 Fc domain that comprises one or more amino acid modifications that alter the pI of the type I-Fc fusion protein. In some embodiments, the type I-Fc fusion protein IgG4 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the type I-Fc fusion protein IgG4 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution

at the position corresponding to K142 of SEQ ID NO: 3500; b) an amino acid substitution at the position corresponding to K221 of SEQ ID NO: 3500; and c) an amino acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 and an amino acid substitution at the position corresponding to K221 of SEQ ID NO: 3500. In some embodiments, the type I-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 (K142E or K142D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K221 of SEQ ID NO: 3500 (K221E or K221D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 (K142E or K142D) and an aspartic acid or glutamic acid substitution at the position corresponding to K221 of SEQ ID NO: 3500 (K221E or K221D).

In certain aspects, a type I:type II heteromultimer of the disclosure comprises an first type I-Fc fusion protein comprising one or more amino acid modifications that increase the pI of the first type I-Fc fusion protein; and a second type I-Fc fusion protein comprising one or more amino acid modifications that decrease the pI of the second type I-Fc fusion protein, wherein the first type I-Fc fusion protein and second type I-Fc fusion protein are different TGFβ superfamily type I receptor polypeptides. For example, a first type I-Fc fusion protein may be modified by substituting one or more neutral or negatively charged amino acids with one or more positively charged amino acids [e.g., an arginine (R), lysine (K), or histidine (H)]. Similarly, a second type I-Fc fusion protein may be modified by substituting one or more neutral or positively charged amino acids with one or more negatively charged amino acids [e.g., aspartic acid (E) or glutamic acid (D)]. In some embodiments, the first type I-Fc fusion protein Fc domain is an IgG1 Fc domain that comprises one or more amino acid modifications that alter the pI of the first type I-Fc fusion protein. In some embodiments, the first type I-Fc fusion protein IgG1 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the first type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N162 of SEQ ID NO: 3100; b) an amino acid substitution at the position corresponding to D179 of SEQ ID NO: 3100; and c) an amino acid substitution at the position corresponding to N162 of SEQ ID NO: 3100 and an amino acid substitution at the position corresponding to D179 of SEQ ID NO: 3100. In some embodiments, the first type I-Fc fusion protein IgG1 Fc domain

comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R, N162K, or N162H); b) an arginine, lysine, or histidine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R, D179K, or D179H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R, N162K. or N162H) and an arginine, lysine, or histidine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R, D179K. or D179H). In some embodiments, the first type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R); b) an arginine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R); and c) an arginine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R) and an arginine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R). In some embodiments, the first type I-Fc fusion protein Fc domain is an IgG2 Fc domain that comprises one or more amino acid modifications that alter the pI of the first type I-Fc fusion protein. In some embodiments, the first type I-Fc fusion protein IgG2 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the first type I-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N160 of SEQ ID NO: 3200; b) an amino acid substitution at the position corresponding to D177 of SEQ ID NO: 3200; and c) an amino acid substitution at the position corresponding to N160 of SEQ ID NO: 3200 and an amino acid substitution at the position corresponding to D177 of SEQ ID NO: 3200. In some embodiments, the first type I-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N160 of SEQ ID NO: 3200 (N160R, N160K, or N160H); b) an arginine, lysine, or histidine substitution at the position corresponding to D177 of SEQ ID NO: 3200 (D177R, D177K, or D177H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N160 of SEQ ID NO: 3200 (N160R, N160K, or N160H) and an arginine, lysine, or histidine substitution at the position corresponding to D177 of SEQ ID NO: 3200 (D177R, D177K. or D177H). In some embodiments, the first type I-Fc fusion protein Fc domain is an IgG3 Fc domain that comprises one or more amino acid modifications that alter the pI of the first type I-Fc fusion protein. In some embodiments, the first type I-Fc fusion protein IgG3 Fc domain

comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3300. In some embodiments, the first type I-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to S169 of SEQ ID NO: 3300; b) an amino acid substitution at the position corresponding to D186 of SEQ ID NO: 3300; and c) an amino acid substitution at the position corresponding to S169 of SEQ ID NO: 3300 and an amino acid substitution at the position corresponding to D186 of SEQ ID NO: 3300. In some embodiments, the first type I-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to S169 of SEQ ID NO: 3300 (S169R, S169K, or S169H); b) an arginine, lysine, or histidine substitution at the position corresponding to D186 of SEQ ID NO: 3300 (D186R, D186K, or D186H); and c) an arginine, lysine, or histidine substitution at the position corresponding to S169 of SEQ ID NO: 3300 (S169R, S169K, or S169H) and an arginine, lysine, or histidine substitution at the position corresponding to D186 of SEQ ID NO: 3300 (D186R, D186K, or D186H). In some embodiments, the first type I-Fc fusion protein Fc domain is an IgG4 Fc domain that comprises one or more amino acid modifications that alter the pI of the first type I-Fc fusion protein. In some embodiments, the first type I-Fc fusion protein IgG4 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the first type I-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N166 of SEQ ID NO: 3500; b) an amino acid substitution at the position corresponding to D183 of SEQ ID NO: 3500; and c) an amino acid substitution at the position corresponding to N166 of SEQ ID NO: 3500 and an amino acid substitution at the position corresponding to D183 of SEQ ID NO: 3500. In some embodiments, the first type I-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N166 of SEQ ID NO: 3500 (N166R, N166K, or N166H); b) an arginine, lysine, or histidine substitution at the position corresponding to D183 of SEQ ID NO: 3500 (D183R, D183K, or D183H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N166 of SEQ ID NO: 3500 (N166R, N166K, or N166H) and an arginine, lysine, or histidine substitution at the position corresponding to D183 of SEQ ID NO: 3500 (D183R, D183K. or D183H). In some embodiments, the second type I-Fc fusion protein Fc domain is an IgG1 Fc

domain that comprises one or more amino acid modifications that alter the pI of the second type I-Fc fusion protein. In some embodiments, the second type I-Fc fusion protein IgG1 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the second type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K138 of SEQ ID NO: 3100; b) an amino acid substitution at the position corresponding to K217 of SEQ ID NO: 3100; and c) an amino acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 and an amino acid substitution at the position corresponding to K217 of SEQ ID NO: 3100. In some embodiments, the second type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E or K138D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217E or K217D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E or K138D) and an aspartic acid or glutamic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217E or K217D). In some embodiments, the second type I-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) a glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E); b) an aspartic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217D); and c) a glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E) and an aspartic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217D). In some embodiments, the second type I-Fc fusion protein Fc domain is an IgG2 Fc domain that comprises one or more amino acid modifications that alter the pI of the second type I-Fc fusion protein. In some embodiments, the second type I-Fc fusion protein IgG2 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the second type I-Fc fusion protein IgG2 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K136 of SEQ ID NO: 3200; b) an amino acid substitution at the position corresponding to K215 of SEQ ID NO: 3200; and c) an amino acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 and an amino acid substitution at the position corresponding to K215 of SEQ ID NO: 3200. In some

embodiments, the second type I-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 (K136E or K136D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K215 of SEQ ID NO: 3200 (K215E or K215D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 (K136E or K136D) and an aspartic acid or glutamic acid substitution at the position corresponding to K215 of SEQ ID NO: 3200 (K215E or K215D). In some embodiments, the second type I-Fc fusion protein Fc domain is an IgG3 Fc domain that comprises one or more amino acid modifications that alter the pI of the second type I-Fc fusion protein. In some embodiments, the second type I-Fc fusion protein IgG3 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3300. In some embodiments, the second type I-Fc fusion protein IgG3 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K145 of SEQ ID NO: 3300; b) an amino acid substitution at the position corresponding to K224 of SEQ ID NO: 3300; and c) an amino acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 and an amino acid substitution at the position corresponding to K224 of SEQ ID NO: 3300. In some embodiments, the second type I-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 (K145E or K145D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K224 of SEQ ID NO: 3300 (K224E or K224D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 (K145E or K145D) and an aspartic acid or glutamic acid substitution at the position corresponding to K224 of SEQ ID NO: 3300 (K224E or K224D). In some embodiments, the second type I-Fc fusion protein Fc domain is an IgG4 Fc domain that comprises one or more amino acid modifications that alter the pI of the second type I-Fc fusion protein. In some embodiments, the second type I-Fc fusion protein IgG4 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the second type I-Fc fusion protein IgG4 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K142 of SEQ ID NO: 3500; b) an amino acid substitution at the position corresponding to K221 of SEQ ID NO: 3500; and c)

an amino acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 and an amino acid substitution at the position corresponding to K221 of SEQ ID NO: 3500. In some embodiments, the second type I-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 (K142E or K142D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K221 of SEQ ID NO: 3500 (K221E or K221D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 (K142E or K142D) and an aspartic acid or glutamic acid substitution at the position corresponding to K221 of SEQ ID NO: 3500 (K221E or K221D).

In certain aspects, a type II:type II heteromultimer of the disclosure comprises an first type II-Fc fusion protein comprising one or more amino acid modifications that increase the pI of the first type II-Fc fusion protein; and a second type II-Fc fusion protein comprising one or more amino acid modifications that decrease the pI of the second type II-Fc fusion protein, wherein the first type II-Fc fusion protein and second type II-Fc fusion protein are different TGFβ superfamily type II receptor polypeptides. For example, a first type II-Fc fusion protein may be modified by substituting one or more neutral or negatively charged amino acids with one or more positively charged amino acids [e.g., an arginine (R), lysine (K), or histidine (H)]. Similarly, a second type II-Fc fusion protein may be modified by substituting one or more neutral or positively charged amino acids with one or more negatively charged amino acids [e.g., aspartic acid (E) or glutamic acid (D)]. In some embodiments, the first type II-Fc fusion protein Fc domain is an IgG1 Fc domain that comprises one or more amino acid modifications that alter the pI of the first type II-Fc fusion protein. In some embodiments, the first type II-Fc fusion protein IgG1 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the first type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N162 of SEQ ID NO: 3100; b) an amino acid substitution at the position corresponding to D179 of SEQ ID NO: 3100; and c) an amino acid substitution at the position corresponding to N162 of SEQ ID NO: 3100 and an amino acid substitution at the position corresponding to D179 of SEQ ID NO: 3100. In some embodiments, the first type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N162 of

SEQ ID NO: 3100 (N162R, N162K, or N162H); b) an arginine, lysine, or histidine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R, D179K, or D179H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R, N162K. or N162H) and an arginine, lysine, or histidine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R, D179K. or D179H). In some embodiments, the first type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R); b) an arginine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R); and c) an arginine substitution at the position corresponding to N162 of SEQ ID NO: 3100 (N162R) and an arginine substitution at the position corresponding to D179 of SEQ ID NO: 3100 (D179R). In some embodiments, the first type II-Fc fusion protein Fc domain is an IgG2 Fc domain that comprises one or more amino acid modifications that alter the pI of the first type II-Fc fusion protein. In some embodiments, the first type II-Fc fusion protein IgG2 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the first type II-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N160 of SEQ ID NO: 3200; b) an amino acid substitution at the position corresponding to D177 of SEQ ID NO: 3200; and c) an amino acid substitution at the position corresponding to N160 of SEQ ID NO: 3200 and an amino acid substitution at the position corresponding to D177 of SEQ ID NO: 3200. In some embodiments, the first type II-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N160 of SEQ ID NO: 3200 (N160R, N160K, or N160H); b) an arginine, lysine, or histidine substitution at the position corresponding to D177 of SEQ ID NO: 3200 (D177R, D177K, or D177H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N160 of SEQ ID NO: 3200 (N160R, N160K, or N160H) and an arginine, lysine, or histidine substitution at the position corresponding to D177 of SEQ ID NO: 3200 (D177R, D177K. or D177H). In some embodiments, the first type II-Fc fusion protein Fc domain is an IgG3 Fc domain that comprises one or more amino acid modifications that alter the pI of the first type II-Fc fusion protein. In some embodiments, the first type II-Fc fusion protein IgG3 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ

ID NO: 3300. In some embodiments, the first type II-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to S169 of SEQ ID NO: 3300; b) an amino acid substitution at the position corresponding to D186 of SEQ ID NO: 3300; and c) an amino acid substitution at the position corresponding to S169 of SEQ ID NO: 3300 and an amino acid substitution at the position corresponding to D186 of SEQ ID NO: 3300. In some embodiments, the first type II-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to S169 of SEQ ID NO: 3300 (S169R, S169K, or S169H); b) an arginine, lysine, or histidine substitution at the position corresponding to D186 of SEQ ID NO: 3300 (D186R, D186K, or D186H); and c) an arginine, lysine, or histidine substitution at the position corresponding to S169 of SEQ ID NO: 3300 (S169R, S169K, or S169H) and an arginine, lysine, or histidine substitution at the position corresponding to D186 of SEQ ID NO: 3300 (D186R, D186K, or D186H). In some embodiments, the first type II-Fc fusion protein Fc domain is an IgG4 Fc domain that comprises one or more amino acid modifications that alter the pI of the first type II-Fc fusion protein. In some embodiments, the first type II-Fc fusion protein IgG4 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the first type II-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to N166 of SEQ ID NO: 3500; b) an amino acid substitution at the position corresponding to D183 of SEQ ID NO: 3500; and c) an amino acid substitution at the position corresponding to N166 of SEQ ID NO: 3500 and an amino acid substitution at the position corresponding to D183 of SEQ ID NO: 3500. In some embodiments, the first type II-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an arginine, lysine, or histidine substitution at the position corresponding to N166 of SEQ ID NO: 3500 (N166R, N166K, or N166H); b) an arginine, lysine, or histidine substitution at the position corresponding to D183 of SEQ ID NO: 3500 (D183R, D183K, or D183H); and c) an arginine, lysine, or histidine substitution at the position corresponding to N166 of SEQ ID NO: 3500 (N166R, N166K, or N166H) and an arginine, lysine, or histidine substitution at the position corresponding to D183 of SEQ ID NO: 3500 (D183R, D183K. or D183H). In some embodiments, the second type II-Fc fusion protein Fc domain is an IgG1 Fc domain that comprises one or more amino acid modifications that alter the pI of the second type II-Fc fusion protein. In some embodiments, the second type II-Fc fusion protein

IgG1 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the second type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K138 of SEQ ID NO: 3100; b) an amino acid substitution at the position corresponding to K217 of SEQ ID NO: 3100; and c) an amino acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 and an amino acid substitution at the position corresponding to K217 of SEQ ID NO: 3100. In some embodiments, the second type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E or K138D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217E or K217D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E or K138D) and an aspartic acid or glutamic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217E or K217D). In some embodiments, the second type II-Fc fusion protein IgG1 Fc domain comprises one or more amino acid substitutions selected from: a) a glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E); b) an aspartic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217D); and c) a glutamic acid substitution at the position corresponding to K138 of SEQ ID NO: 3100 (K138E) and an aspartic acid substitution at the position corresponding to K217 of SEQ ID NO: 3100 (K217D). In some embodiments, the second type II-Fc fusion protein Fc domain is an IgG2 Fc domain that comprises one or more amino acid modifications that alter the pI of the second type II-Fc fusion protein. In some embodiments, the second type II-Fc fusion protein IgG2 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the second type I-Fc fusion protein IgG2 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K136 of SEQ ID NO: 3200; b) an amino acid substitution at the position corresponding to K215 of SEQ ID NO: 3200; and c) an amino acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 and an amino acid substitution at the position corresponding to K215 of SEQ ID NO: 3200. In some embodiments, the second type II-Fc fusion protein IgG2 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic

acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 (K136E or K136D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K215 of SEQ ID NO: 3200 (K215E or K215D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K136 of SEQ ID NO: 3200 (K136E or K136D) and an aspartic acid or glutamic acid substitution at the position corresponding to K215 of SEQ ID NO: 3200 (K215E or K215D). In some embodiments, the second type II-Fc fusion protein Fc domain is an IgG3 Fc domain that comprises one or more amino acid modifications that alter the pI of the second type II-Fc fusion protein. In some embodiments, the second type II-Fc fusion protein IgG3 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3300. In some embodiments, the second type II-Fc fusion protein IgG3 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K145 of SEQ ID NO: 3300; b) an amino acid substitution at the position corresponding to K224 of SEQ ID NO: 3300; and c) an amino acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 and an amino acid substitution at the position corresponding to K224 of SEQ ID NO: 3300. In some embodiments, the second type II-Fc fusion protein IgG3 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 (K145E or K145D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K224 of SEQ ID NO: 3300 (K224E or K224D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K145 of SEQ ID NO: 3300 (K145E or K145D) and an aspartic acid or glutamic acid substitution at the position corresponding to K224 of SEQ ID NO: 3300 (K224E or K224D). In some embodiments, the second type II-Fc fusion protein Fc domain is an IgG4 Fc domain that comprises one or more amino acid modifications that alter the pI of the second type II-Fc fusion protein. In some embodiments, the second type II-Fc fusion protein IgG4 Fc domain comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the second type II-Fc fusion protein IgG4 fusion Fc domain comprises one or more amino acid substitutions selected from: a) an amino acid substitution at the position corresponding to K142 of SEQ ID NO: 3500; b) an amino acid substitution at the position corresponding to K221 of SEQ ID NO: 3500; and c) an amino acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 and an amino acid substitution at the

position corresponding to K221 of SEQ ID NO: 3500. In some embodiments, the second type II-Fc fusion protein IgG4 Fc domain comprises one or more amino acid substitutions selected from: a) an aspartic acid or glutamic acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 (K142E or K142D); b) an aspartic acid or glutamic acid substitution at the position corresponding to K221 of SEQ ID NO: 3500 (K221E or K221D); and c) an aspartic acid or glutamic acid substitution at the position corresponding to K142 of SEQ ID NO: 3500 (K142E or K142D) and an aspartic acid or glutamic acid substitution at the position corresponding to K221 of SEQ ID NO: 3500 (K221E or K221D).

As described herein, type I-Fc fusion proteins and/or type II-Fc fusion proteins may comprise one or more modifications that promote heteromultimer formation (e.g., type I-Fc:type II-Fc heterodimerization). Similarly, type I-Fc fusion proteins and/or type II-Fc fusion proteins may comprise one or more modifications that inhibit homomultimer formation (e.g., type I-Fc and/or type II-Fc homodimerization). In some embodiments, type I-Fc fusion proteins and/or type II-Fc fusion proteins may comprise one or more modifications that promote heteromultimer formation and comprise one or more modifications that inhibit homomultimer formation.

For example, in some embodiments, an type I:type II heteromultimer comprises: a) a type I-Fc fusion protein having an IgG1 Fc domain comprising a cysteine substitution at position S132 of SEQ ID NO: 3100 (S132C) and a tryptophan substitution at position T144 of SEQ ID NO: 3100 (T144W); and b) an type II-Fc fusion protein having an IgG1 Fc domain comprising a cysteine substitution at position Y127 of SEQ ID NO: 3100 (Y127C), a serine substitution at position T144 of SEQ ID NO: 3100 (T144S), an alanine substitution at position L146 of SEQ ID NO: 3100 (L146A), and a valine substitution at position Y185 of SEQ ID NO: 3100 (Y185V). In some embodiments, an type I:type II heteromultimer comprises: a) an type II-Fc fusion protein having an IgG1 Fc domain comprising a cysteine substitution at position S132 of SEQ ID NO: 3100 (S132C) and a tryptophan substitution at position T144 of SEQ ID NO: 3100 (T144W); and b) an type I-Fc fusion protein having an IgG1 Fc domain comprising a cysteine substitution at position Y127 of SEQ ID NO: 3100 (Y127C), a serine substitution at position T144 of SEQ ID NO: 3100 (T144S), an alanine substitution at position L146 of SEQ ID NO: 3100 (L146A), and a valine substitution at position Y185 of SEQ ID NO: 3100 (Y185V). In some embodiments, a type I:type II heteromultimer comprises: a) an type I-Fc fusion protein having an IgG2 Fc domain comprising a cysteine substitution at position S130 of SEQ ID NO: 3200 (S130C) and a tryptophan substitution at position T142 of SEQ ID NO: 3200 (T142W); and b) an type II-Fc

fusion protein having an IgG2 Fc domain comprising a cysteine substitution at position Y125 of SEQ ID NO: 3200 (Y125C), a serine substitution at position T142 of SEQ ID NO: 3200 (T142S), an alanine substitution at position L144 of SEQ ID NO: 3200 (L144A), and a valine substitution at position Y183 of SEQ ID NO: 3200 (Y183V). In some embodiments, an type I:type II heteromultimer comprises: a) an type II-Fc fusion protein having an IgG2 Fc domain comprising a cysteine substitution at position S130 of SEQ ID NO: 3200 (S130C) and a tryptophan substitution at position T142 of SEQ ID NO: 3200 (T142W); and b) an type I-Fc fusion protein having an IgG2 Fc domain comprising a cysteine substitution at position Y125 of SEQ ID NO: 3200 (Y125C), a serine substitution at position T142 of SEQ ID NO: 3200 (T142S), an alanine substitution at position L144 of SEQ ID NO: 3200 (L144A), and a valine substitution at position Y183 of SEQ ID NO: 3200 (Y183V). In some embodiments, an type I:type II heteromultimer comprises: a) an type I-Fc fusion protein having an IgG3 Fc domain comprising a cysteine substitution at position S139 of SEQ ID NO: 3300 (S139C) and a tryptophan substitution at position T151 of SEQ ID NO: 3300 (T151W); and b) the type II-Fc fusion protein having an IgG3 Fc domain comprising a cysteine substitution at position Y134 of SEQ ID NO: 3300 (Y134C), a serine substitution at position T151 of SEQ ID NO: 3300 (T151S), an alanine substitution at position L153 of SEQ ID NO: 3300 (L153A), and a valine substitution at position Y192 of SEQ ID NO: 3300 (Y192V). In some embodiments, an type I:type II heteromultimer comprises: a) an type II-Fc fusion protein having an IgG3 Fc domain comprising a cysteine substitution at position S139 of SEQ ID NO: 3300 (S139C) and a tryptophan substitution at position T151 of SEQ ID NO: 3300 (T151W); and b) an type I-Fc fusion protein having an IgG3 Fc domain comprising a cysteine substitution at position Y134 of SEQ ID NO: 3300 (Y134C), a serine substitution at position T151 of SEQ ID NO: 3300 (T151S), an alanine substitution at position L153 of SEQ ID NO: 3300 (L153A), and a valine substitution at position Y192 of SEQ ID NO: 3300 (Y192V). In some embodiments, an type I:type II heteromultimer comprises: a) an type I-Fc fusion protein having an IgG4 Fc domain comprises a cysteine substitution at position S136 of SEQ ID NO: 3500 (S136C) and a tryptophan substitution at position T148 of SEQ ID NO: 3500 (T148W); and b) an typeII-Fc fusion protein having an IgG4 Fc domain comprises a cysteine substitution at position Y131 of SEQ ID NO: 3500 (Y131C), a serine substitution at position T148 of SEQ ID NO: 3500 (T148S), an alanine substitution at position L150 of SEQ ID NO: 3500 (L150A), and a valine substitution at position Y189 of SEQ ID NO: 3500 (Y189V). In some embodiments, an type I:type II heteromultimer comprises: a) an type II-Fc fusion protein having an IgG4 Fc domain comprising a cysteine substitution at position S136 of SEQ ID NO: 3500 (S136C) and a

tryptophan substitution at position T148 of SEQ ID NO: 3500 (T148W); and b) an type I-Fc fusion protein having an IgG4 Fc domain comprising a cysteine substitution at position Y131 of SEQ ID NO: 3500 (Y131C), a serine substitution at position T148 of SEQ ID NO: 3500 (T148S), an alanine substitution at position L150 of SEQ ID NO: 3500 (L150A), and a valine substitution at position Y189 of SEQ ID NO: 3500 (Y189V).

In some embodiments, an type I:type I heteromultimer comprises: a) a first type I-Fc fusion protein having an IgG1 Fc domain comprising a cysteine substitution at position S132 of SEQ ID NO: 3100 (S132C) and a tryptophan substitution at position T144 of SEQ ID NO: 3100 (T144W); and b) an secpmd type I-Fc fusion protein having an IgG1 Fc domain comprising a cysteine substitution at position Y127 of SEQ ID NO: 3100 (Y127C), a serine substitution at position T144 of SEQ ID NO: 3100 (T144S), an alanine substitution at position L146 of SEQ ID NO: 3100 (L146A), and a valine substitution at position Y185 of SEQ ID NO: 3100 (Y185V). In some embodiments, a type I:type I heteromultimer comprises: a) an first type I-Fc fusion protein having an IgG2 Fc domain comprising a cysteine substitution at position S130 of SEQ ID NO: 3200 (S130C) and a tryptophan substitution at position T142 of SEQ ID NO: 3200 (T142W); and b) a second type I-Fc fusion protein having an IgG2 Fc domain comprising a cysteine substitution at position Y125 of SEQ ID NO: 3200 (Y125C), a serine substitution at position T142 of SEQ ID NO: 3200 (T142S), an alanine substitution at position L144 of SEQ ID NO: 3200 (L144A), and a valine substitution at position Y183 of SEQ ID NO: 3200 (Y183V). In some embodiments, a type I:type I heteromultimer comprises: a) a first type I-Fc fusion protein having an IgG3 Fc domain comprising a cysteine substitution at position S139 of SEQ ID NO: 3300 (S139C) and a tryptophan substitution at position T151 of SEQ ID NO: 3300 (T151W); and b) a second type I-Fc fusion protein having an IgG3 Fc domain comprising a cysteine substitution at position Y134 of SEQ ID NO: 3300 (Y134C), a serine substitution at position T151 of SEQ ID NO: 3300 (T151S), an alanine substitution at position L153 of SEQ ID NO: 3300 (L153A), and a valine substitution at position Y192 of SEQ ID NO: 3300 (Y192V). In some embodiments, a type I:type I heteromultimer comprises: a) a first type I-Fc fusion protein having an IgG4 Fc domain comprises a cysteine substitution at position S136 of SEQ ID NO: 3500 (S136C) and a tryptophan substitution at position T148 of SEQ ID NO: 3500 (T148W); and b) a seond type I-Fc fusion protein having an IgG4 Fc domain comprises a cysteine substitution at position Y131 of SEQ ID NO: 3500 (Y131C), a serine substitution at position T148 of SEQ ID NO: 3500 (T148S), an alanine substitution at position L150 of SEQ ID NO: 3500 (L150A), and a valine substitution at position Y189 of SEQ ID NO: 3500 (Y189V).

In some embodiments, a type II:type II heteromultimer comprises: a) a first type II-Fc fusion protein having an IgG1 Fc domain comprising a cysteine substitution at position S132 of SEQ ID NO: 3100 (S132C) and a tryptophan substitution at position T144 of SEQ ID NO: 3100 (T144W); and b) an secpmd type II-Fc fusion protein having an IgG1 Fc domain comprising a cysteine substitution at position Y127 of SEQ ID NO: 3100 (Y127C), a serine substitution at position T144 of SEQ ID NO: 3100 (T144S), an alanine substitution at position L146 of SEQ ID NO: 3100 (L146A), and a valine substitution at position Y185 of SEQ ID NO: 3100 (Y185V). In some embodiments, a type II:type II heteromultimer comprises: a) an first type II-Fc fusion protein having an IgG2 Fc domain comprising a cysteine substitution at position S130 of SEQ ID NO: 3200 (S130C) and a tryptophan substitution at position T142 of SEQ ID NO: 3200 (T142W); and b) a second type II-Fc fusion protein having an IgG2 Fc domain comprising a cysteine substitution at position Y125 of SEQ ID NO: 3200 (Y125C), a serine substitution at position T142 of SEQ ID NO: 3200 (T142S), an alanine substitution at position L144 of SEQ ID NO: 3200 (L144A), and a valine substitution at position Y183 of SEQ ID NO: 3200 (Y183V). In some embodiments, a type I:type I heteromultimer comprises: a) a first type II-Fc fusion protein having an IgG3 Fc domain comprising a cysteine substitution at position S139 of SEQ ID NO: 3300 (S139C) and a tryptophan substitution at position T151 of SEQ ID NO: 3300 (T151W); and b) a second type II-Fc fusion protein having an IgG3 Fc domain comprising a cysteine substitution at position Y134 of SEQ ID NO: 3300 (Y134C), a serine substitution at position T151 of SEQ ID NO: 3300 (T151S), an alanine substitution at position L153 of SEQ ID NO: 3300 (L153A), and a valine substitution at position Y192 of SEQ ID NO: 3300 (Y192V). In some embodiments, a type II:type II heteromultimer comprises: a) a first type II-Fc fusion protein having an IgG4 Fc domain comprises a cysteine substitution at position S136 of SEQ ID NO: 3500 (S136C) and a tryptophan substitution at position T148 of SEQ ID NO: 3500 (T148W); and b) a seond type II-Fc fusion protein having an IgG4 Fc domain comprises a cysteine substitution at position Y131 of SEQ ID NO: 3500 (Y131C), a serine substitution at position T148 of SEQ ID NO: 3500 (T148S), an alanine substitution at position L150 of SEQ ID NO: 3500 (L150A), and a valine substitution at position Y189 of SEQ ID NO: 3500 (Y189V).

In certain aspects, a type I:type II heteromultimer of the disclosure comprises: a) an type I-Fc fusion protein having an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 660; and b) a type II-Fc fusion protein having an Fc domain that is at least 75%,

80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 670. In some embodiments, the type I-Fc fusion protein Fc domain comprises one or more amino acid substitutions selected from: a) a glutamic acid at the position corresponding to 138 of SEQ ID NO: 660; b) an aspartic acid at the position corresponding to 217 of SEQ ID NO: 660; and c) a glutamic acid at the position corresponding to 138 of SEQ ID NO: 660 and an aspartic acid at the position corresponding to 217 of SEQ ID NO: 660. Optionally, the type I-Fc fusion protein Fc domain further comprises a cysteine at the position corresponding to 132 of SEQ ID NO: 660 and a tryptophan at the position corresponding to 144 of SEQ ID NO: 660. In some embodiments, the type II-Fc fusion protein Fc domain comprises one or more amino acid substitutions selected from: a) an arginine at the position corresponding to 162 of SEQ ID NO: 670; b) an arginine at the position corresponding to 179 of SEQ ID NO: 670; and c) an arginine at the position corresponding to 162 of SEQ ID NO: 670 and an arginine at the position corresponding to 179 of SEQ ID NO: 670. Optionally, the type II-Fc fusion protein Fc domain further comprises a cysteine at the position corresponding to 127 of SEQ ID NO: 670, a serine at the position corresponding to 144 of SEQ ID NO: 670, an alanine at the position corresponding to 146 of SEQ ID NO: 670, and a valine at the position corresponding to 185 of SEQ ID NO: 670.

In certain aspects, a type I:type II heteromultimer of the disclosure comprises: a) a type II-Fc fusion protein having an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 660; and b) a type I-Fc fusion protein having an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 670. In some embodiments, the type II-Fc fusion protein Fc domain comprises one or more amino acid substitutions selected from: a) a glutamic acid at the position corresponding to 138 of SEQ ID NO: 660; b) an aspartic acid at the position corresponding to 217 of SEQ ID NO: 660; and c) a glutamic acid at the position corresponding to 138 of SEQ ID NO: 660 and an aspartic acid at the position corresponding to 217 of SEQ ID NO: 660. Optionally, the type II-Fc fusion protein Fc domain further comprises a cysteine at the position corresponding to 132 of SEQ ID NO: 660 and a tryptophan at the position corresponding to 144 of SEQ ID NO: 660. In some embodiments, the type I-Fc fusion protein Fc domain comprises one or more amino acid substitutions selected from: a) an arginine at the position corresponding to 162 of SEQ ID NO: 670; b) an arginine at the position corresponding to 179 of SEQ ID NO: 670; and c) an arginine at the

position corresponding to 162 of SEQ ID NO: 670 and an arginine at the position corresponding to 179 of SEQ ID NO: 670. Optionally, the type I-Fc fusion protein Fc domain further comprises a cysteine at the position corresponding to 127 of SEQ ID NO: 670, a serine at the position corresponding to 144 of SEQ ID NO: 670, an alanine at the position corresponding to 146 of SEQ ID NO: 670, and a valine at the position corresponding to 185 of SEQ ID NO: 670.

In certain aspects, a type I:type I heteromultimer of the disclosure comprises: a) a first type I-Fc fusion protein having an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 660; and b) a second type I-Fc fusion protein having an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 670. In some embodiments, the first type I-Fc fusion protein Fc domain comprises one or more amino acid substitutions selected from: a) a glutamic acid at the position corresponding to 138 of SEQ ID NO: 660; b) an aspartic acid at the position corresponding to 217 of SEQ ID NO: 660; and c) a glutamic acid at the position corresponding to 138 of SEQ ID NO: 660 and an aspartic acid at the position corresponding to 217 of SEQ ID NO: 660. Optionally, the first type I-Fc fusion protein Fc domain further comprises a cysteine at the position corresponding to 132 of SEQ ID NO: 660 and a tryptophan at the position corresponding to 144 of SEQ ID NO: 660. In some embodiments, the second type I-Fc fusion protein Fc domain comprises one or more amino acid substitutions selected from: a) an arginine at the position corresponding to 162 of SEQ ID NO: 670; b) an arginine at the position corresponding to 179 of SEQ ID NO: 670; and c) an arginine at the position corresponding to 162 of SEQ ID NO: 670 and an arginine at the position corresponding to 179 of SEQ ID NO: 670. Optionally, the second type I-Fc fusion protein Fc domain further comprises a cysteine at the position corresponding to 127 of SEQ ID NO: 670, a serine at the position corresponding to 144 of SEQ ID NO: 670, an alanine at the position corresponding to 146 of SEQ ID NO: 670, and a valine at the position corresponding to 185 of SEQ ID NO: 670.

In certain aspects, a type II:type II heteromultimer of the disclosure comprises: a) a first type II-Fc fusion protein having an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 660; and b) a second type II-Fc fusion protein having an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 670. In some embodiments, the first

type II-Fc fusion protein Fc domain comprises one or more amino acid substitutions selected from: a) a glutamic acid at the position corresponding to 138 of SEQ ID NO: 660; b) an aspartic acid at the position corresponding to 217 of SEQ ID NO: 660; and c) a glutamic acid at the position corresponding to 138 of SEQ ID NO: 660 and an aspartic acid at the position corresponding to 217 of SEQ ID NO: 660. Optionally, the first type II-Fc fusion protein Fc domain further comprises a cysteine at the position corresponding to 132 of SEQ ID NO: 660 and a tryptophan at the position corresponding to 144 of SEQ ID NO: 660. In some embodiments, the second type II-Fc fusion protein Fc domain comprises one or more amino acid substitutions selected from: a) an arginine at the position corresponding to 162 of SEQ ID NO: 670; b) an arginine at the position corresponding to 179 of SEQ ID NO: 670; and c) an arginine at the position corresponding to 162 of SEQ ID NO: 670 and an arginine at the position corresponding to 179 of SEQ ID NO: 670. Optionally, the second type II-Fc fusion protein Fc domain further comprises a cysteine at the position corresponding to 127 of SEQ ID NO: 670, a serine at the position corresponding to 144 of SEQ ID NO: 670, an alanine at the position corresponding to 146 of SEQ ID NO: 670, and a valine at the position corresponding to 185 of SEQ ID NO: 670.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type I-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to S132 of SEQ ID NO: 3100 (S132C), a tryptophan at the position corresponding to T144 of SEQ ID NO: 3100 (T144W), and an acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100; and b) the type II-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), and a valine at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V). In some embodiments, wherein the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein : a) the type II-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to S132 of SEQ ID NO: 3100 (S132C), a tryptophan at the position corresponding to T144 of SEQ ID NO: 3100 (T144W), and an acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100; and b) the type I-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), and a valine at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V). In some embodiments, wherein the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100.

In certain aspects, the disclosure relates to a recombinant type I:type I heteromultimer comprising at least a first type I-Fc fusion protein and a second type I-Fc fusion protein, wherein: a) the first type I-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to S132 of SEQ ID NO: 3100 (S132C), a tryptophan at the position corresponding to T144 of SEQ ID NO: 3100 (T144W), and an acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100; and b) the second type I-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), and a valine at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V). In some embodiments, wherein the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is a glutamic acid. In some embodiments, the first type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the second type I-Fc

fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100.

In certain aspects, the disclosure relates to a recombinant type II:type II heteromultimer comprising at least a first type II-Fc fusion protein and a second type II-Fc fusion protein, wherein: a) the first type II-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to S132 of SEQ ID NO: 3100 (S132C), a tryptophan at the position corresponding to T144 of SEQ ID NO: 3100 (T144W), and an acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100; and b) the second type II-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), and a valine at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V). In some embodiments, wherein the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is a glutamic acid. In some embodiments, the first type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the second type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type I-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to S132 of SEQ ID NO: 3100 (S132C), and a tryptophan at the position corresponding to T144 of SEQ ID NO: 3100 (T144W); and b) the type II-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), a valine at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V), and an acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100. In some embodiments, wherein the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H213 of SEQ ID

NO: 3100 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type II-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to S132 of SEQ ID NO: 3100 (S132C), and a tryptophan at the position corresponding to T144 of SEQ ID NO: 3100 (T144W); and b) the type I-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), and a valine at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V), and an acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100. In some embodiments, wherein the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100.

In certain aspects, the disclosure relates to a recombinant type I:type I heteromultimer comprising at least one first type I-Fc fusion protein and a second type I-Fc fusion protein, wherein: a) the first type I-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to S132 of SEQ ID NO: 3100 (S132C), and a tryptophan at the position corresponding to T144 of SEQ ID NO: 3100 (T144W); and b) the second type I-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), a valine at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V), and an acidic amino acid at the position

corresponding to H213 of SEQ ID NO: 3100. In some embodiments, wherein the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is a glutamic acid. In some embodiments, the first type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the second type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100.

In certain aspects, the disclosure relates to a recombinant type II:type II heteromultimer comprising at least one first type II-Fc fusion protein and a second type II-Fc fusion protein, wherein: a) the first type II-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to S132 of SEQ ID NO: 3100 (S132C), and a tryptophan at the position corresponding to T144 of SEQ ID NO: 3100 (T144W); and b) the second type II-Fc fusion protein comprises an IgG1 Fc domain comprising a cysteine at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), a valine at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V), and an acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100. In some embodiments, wherein the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H213 of SEQ ID NO: 3100 is a glutamic acid. In some embodiments, the first type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100. In some embodiments, the second type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3100.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type I-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to S130 of SEQ ID NO: 3200 (S130C), a tryptophan at the position corresponding to T142 of SEQ ID NO: 3200 (T142W), and an acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200; and b) the

type II-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to Y125 of SEQ ID NO: 3200 (Y125C), a serine at the position corresponding to T142 of SEQ ID NO: 3200 (T142S), an alanine at the position corresponding to L144 of SEQ ID NO: 3200 (L144A), and a valine at the position corresponding to Y183 of SEQ ID NO: 3200 (Y183V). In some embodiments, wherein the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type II-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to S130 of SEQ ID NO: 3200 (S130C), a tryptophan at the position corresponding to T142 of SEQ ID NO: 3200 (T142W), and an acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200; and b) the type I-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to Y125 of SEQ ID NO: 3200 (Y125C), a serine at the position corresponding to T142 of SEQ ID NO: 3200 (T142S), an alanine at the position corresponding to L144 of SEQ ID NO: 3200 (L144A), and a valine at the position corresponding to Y183 of SEQ ID NO: 3200 (Y183V). In some embodiments, wherein the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200.

In certain aspects, the disclosure relates to a recombinant type I:type I heteromultimer comprising at first type I-Fc fusion protein and a second type I-Fc fusion protein, wherein: a) the first type I-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to S130 of SEQ ID NO: 3200 (S130C), a tryptophan at the position

corresponding to T142 of SEQ ID NO: 3200 (T142W), and an acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200; and b) the second type I-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to Y125 of SEQ ID NO: 3200 (Y125C), a serine at the position corresponding to T142 of SEQ ID NO: 3200 (T142S), an alanine at the position corresponding to L144 of SEQ ID NO: 3200 (L144A), and a valine at the position corresponding to Y183 of SEQ ID NO: 3200 (Y183V). In some embodiments, wherein the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is a glutamic acid. In some embodiments, the first type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the second type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200.

In certain aspects, the disclosure relates to a recombinant type II:type II heteromultimer comprising at first type II-Fc fusion protein and a second type II-Fc fusion protein, wherein: a) the first type II-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to S130 of SEQ ID NO: 3200 (S130C), a tryptophan at the position corresponding to T142 of SEQ ID NO: 3200 (T142W), and an acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200; and b) the second type II-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to Y125 of SEQ ID NO: 3200 (Y125C), a serine at the position corresponding to T142 of SEQ ID NO: 3200 (T142S), an alanine at the position corresponding to L144 of SEQ ID NO: 3200 (L144A), and a valine at the position corresponding to Y183 of SEQ ID NO: 3200 (Y183V). In some embodiments, wherein the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is a glutamic acid. In some embodiments, the first type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the second type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein : a) the type I-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to S130 of SEQ ID NO: 3200 (S130C), and a tryptophan at the position corresponding to T142 of SEQ ID NO: 3200 (T142W); and b) the type II-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to Y125 of SEQ ID NO: 3200 (Y125C), a serine at the position corresponding to T142 of SEQ ID NO: 3200 (T142S), an alanine at the position corresponding to L144 of SEQ ID NO: 3200 (L144A), a valine at the position corresponding to Y183 of SEQ ID NO: 3200 (Y183V), and an acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200. In some embodiments, wherein the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type II-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to S130 of SEQ ID NO: 3200 (S130C), and a tryptophan at the position corresponding to T142 of SEQ ID NO: 3200 (T142W); and b) the type I-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to Y125 of SEQ ID NO: 3200 (Y125C), a serine at the position corresponding to T142 of SEQ ID NO: 3200 (T142S), an alanine at the position corresponding to L144 of SEQ ID NO: 3200 (L144A), a valine at the position corresponding to Y183 of SEQ ID NO: 3200 (Y183V), and an acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200. In some embodiments, wherein the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the type

II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200.

In certain aspects, the disclosure relates to a recombinant type I:type I heteromultimer comprising a first type I-Fc fusion protein and a second type I-Fc fusion protein, wherein : a) the first type I-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to S130 of SEQ ID NO: 3200 (S130C), and a tryptophan at the position corresponding to T142 of SEQ ID NO: 3200 (T142W); and b) the second type I-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to Y125 of SEQ ID NO: 3200 (Y125C), a serine at the position corresponding to T142 of SEQ ID NO: 3200 (T142S), an alanine at the position corresponding to L144 of SEQ ID NO: 3200 (L144A), a valine at the position corresponding to Y183 of SEQ ID NO: 3200 (Y183V), and an acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200. In some embodiments, wherein the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is a glutamic acid. In some embodiments, the first type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the second type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200.

In certain aspects, the disclosure relates to a recombinant type II:type II heteromultimer comprising a first type II-Fc fusion protein and a second type II-Fc fusion protein, wherein : a) the first type II-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to S130 of SEQ ID NO: 3200 (S130C), and a tryptophan at the position corresponding to T142 of SEQ ID NO: 3200 (T142W); and b) the second type II-Fc fusion protein comprises an IgG2 Fc domain comprising a cysteine at the position corresponding to Y125 of SEQ ID NO: 3200 (Y125C), a serine at the position corresponding to T142 of SEQ ID NO: 3200 (T142S), an alanine at the position corresponding to L144 of SEQ ID NO: 3200 (L144A), a valine at the position corresponding to Y183 of SEQ ID NO: 3200 (Y183V), and an acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200. In some embodiments, wherein the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H211 of SEQ ID NO: 3200 is a glutamic acid. In some embodiments, the first type II-Fc fusion protein Fc

domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200. In some embodiments, the second type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3200.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type I-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to S136 of SEQ ID NO: 3500 (S136C), a tryptophan at the position corresponding to T148 of SEQ ID NO: 3500 (T148W), and an acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500; and b) the type II-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to Y131 of SEQ ID NO: 3500 (Y131C), a serine at the position corresponding to T148 of SEQ ID NO: 3500 (T148S), an alanine at the position corresponding to L150 of SEQ ID NO: 3500 (L150A), and a valine at the position corresponding to Y189 of SEQ ID NO: 3500 (Y189V). In some embodiments, wherein the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500.

In certain aspects, the disclosure relates to a recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type II-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to S136 of SEQ ID NO: 3500 (S136C), a tryptophan at the position corresponding to T148 of SEQ ID NO: 3500 (T148W), and an acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500; and b) the type I-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to Y131 of SEQ ID NO: 3500 (Y131C), a serine at the position corresponding to T148 of SEQ ID NO: 3500 (T148S), an alanine at the position corresponding to L150 of SEQ ID NO: 3500 (L150A), and a valine at the position corresponding to Y189 of SEQ ID NO: 3500 (Y189V). In some embodiments, wherein the acidic amino acid at the position

corresponding to H217 of SEQ ID NO: 3500 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500.

In certain aspects, the disclosure relates to a recombinant type I:type I heteromultimer comprising a first type I-Fc fusion protein and a second type I-Fc fusion protein, wherein: a) the first type I-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to S136 of SEQ ID NO: 3500 (S136C), a tryptophan at the position corresponding to T148 of SEQ ID NO: 3500 (T148W), and an acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500; and b) the second type I-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to Y131 of SEQ ID NO: 3500 (Y131C), a serine at the position corresponding to T148 of SEQ ID NO: 3500 (T148S), an alanine at the position corresponding to L150 of SEQ ID NO: 3500 (L150A), and a valine at the position corresponding to Y189 of SEQ ID NO: 3500 (Y189V). In some embodiments, wherein the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is a glutamic acid. In some embodiments, the first type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the second type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500.

In certain aspects, the disclosure relates to a recombinant type II:type II heteromultimer comprising a first type II-Fc fusion protein and a second type II-Fc fusion protein, wherein: a) the first type II-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to S136 of SEQ ID NO: 3500 (S136C), a tryptophan at the position corresponding to T148 of SEQ ID NO: 3500 (T148W), and an acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500; and b) the second type II-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to Y131 of SEQ ID NO: 3500 (Y131C), a serine at the position corresponding to T148 of SEQ ID NO: 3500 (T148S), an alanine at the position

corresponding to L150 of SEQ ID NO: 3500 (L150A), and a valine at the position corresponding to Y189 of SEQ ID NO: 3500 (Y189V). In some embodiments, wherein the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is a glutamic acid. In some embodiments, the first type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the second type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500.

In certain aspects, the disclosure relates to recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type I-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to S136 of SEQ ID NO: 3500 (S136C), and a tryptophan at the position corresponding to T148 of SEQ ID NO: 3500 (T148W); and b) the type II-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to Y131 of SEQ ID NO: 3500 (Y131C), a serine at the position corresponding to T148 of SEQ ID NO: 3500 (T148S), an alanine at the position corresponding to L150 of SEQ ID NO: 3500 (L150A), a valine at the position corresponding to Y189 of SEQ ID NO: 3500 (Y189V), and an acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500. In some embodiments, wherein the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500.

In certain aspects, the disclosure relates to recombinant type I:type II heteromultimer comprising at least one type I-Fc fusion protein and at least one type II-Fc fusion protein, wherein: a) the type II-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to S136 of SEQ ID NO: 3500 (S136C), and a tryptophan at the position corresponding to T148 of SEQ ID NO: 3500 (T148W); and b) the type I-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to

Y131 of SEQ ID NO: 3500 (Y131C), a serine at the position corresponding to T148 of SEQ ID NO: 3500 (T148S), an alanine at the position corresponding to L150 of SEQ ID NO: 3500 (L150A), a valine at the position corresponding to Y189 of SEQ ID NO: 3500 (Y189V), and an acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500. In some embodiments, wherein the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is a glutamic acid. In some embodiments, the type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500.

In certain aspects, the disclosure relates to recombinant type I:type I heteromultimer comprising a first type I-Fc fusion protein and a second type I-Fc fusion protein, wherein: a) the first type I-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to S136 of SEQ ID NO: 3500 (S136C), and a tryptophan at the position corresponding to T148 of SEQ ID NO: 3500 (T148W); and b) the second type I-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to Y131 of SEQ ID NO: 3500 (Y131C), a serine at the position corresponding to T148 of SEQ ID NO: 3500 (T148S), an alanine at the position corresponding to L150 of SEQ ID NO: 3500 (L150A), a valine at the position corresponding to Y189 of SEQ ID NO: 3500 (Y189V), and an acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500. In some embodiments, wherein the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is a glutamic acid. In some embodiments, the first type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the second type I-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500.

In certain aspects, the disclosure relates to recombinant type II:type II heteromultimer comprising a first type II-Fc fusion protein and a second type II-Fc fusion protein, wherein: a) the first type II-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to S136 of SEQ ID NO: 3500 (S136C), and a tryptophan at the

position corresponding to T148 of SEQ ID NO: 3500 (T148W); and b) the second type II-Fc fusion protein comprises an IgG4 Fc domain comprising a cysteine at the position corresponding to Y131 of SEQ ID NO: 3500 (Y131C), a serine at the position corresponding to T148 of SEQ ID NO: 3500 (T148S), an alanine at the position corresponding to L150 of SEQ ID NO: 3500 (L150A), a valine at the position corresponding to Y189 of SEQ ID NO: 3500 (Y189V), and an acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500. In some embodiments, wherein the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is an aspartic acid. In some embodiments, the acidic amino acid at the position corresponding to H217 of SEQ ID NO: 3500 is a glutamic acid. In some embodiments, the first type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500. In some embodiments, the second type II-Fc fusion protein Fc domain is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to the amino acid sequence of SEQ ID NO: 3500.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one ActRIIA-Fc fusion protein. In some embodiments, an ALK1-Fc:ActRIIA-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ActRIIA-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ActRIIA-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one ActRIIA-Fc fusion protein. In some embodiments, an ALK2-Fc:ActRIIA-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ActRIIA-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ActRIIA-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one ActRIIA-Fc fusion protein. In some embodiments, an ALK3-Fc:ActRIIA-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ActRIIA-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily

ligands such as those described herein. In some embodiments, an ALK3-Fc:ActRIIA-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK4-Fc fusion protein and at least one ActRIIA-Fc fusion protein. In some embodiments, an ALK4-Fc:ActRIIA-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ActRIIA-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ActRIIA-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK5-Fc fusion protein and at least one ActRIIA-Fc fusion protein. In some embodiments, an ALK5-Fc:ActRIIA-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:ActRIIA-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:ActRIIA-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK6-Fc fusion protein and at least one ActRIIA-Fc fusion protein. In some embodiments, an ALK6-Fc:ActRIIA-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:ActRIIA-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:ActRIIA-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK7-Fc fusion protein and at least one ActRIIA-Fc fusion protein. In some embodiments, an ALK7-Fc:ActRIIA-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:ActRIIA-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:ActRIIA-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some embodiments, an ALK1-Fc:ActRIIB-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-

Fc:ActRIIB-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ActRIIB-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some embodiments, an ALK2-Fc:ActRIIB-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ActRIIB-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ActRIIB-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some embodiments, an ALK3-Fc:ActRIIB-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ActRIIB-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ActRIIB-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK4-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some embodiments, an ALK4-Fc:ActRIIB-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ActRIIB-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ActRIIB-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK5-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some embodiments, an ALK5-Fc:ActRIIB-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:ActRIIB-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:ActRIIB-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK6-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some embodiments, an ALK6-Fc:ActRIIB-Fc heteromultimers binds to one or more TGF-beta

superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:ActRIIB-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:ActRIIB-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK7-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some embodiments, an ALK7-Fc:ActRIIB-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:ActRIIB-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:ActRIIB-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some embodiments, an ALK1-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some embodiments, an ALK2-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some embodiments, an ALK3-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK4-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some

embodiments, an ALK4-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK5-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some embodiments, an ALK5-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK6-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some embodiments, an ALK6-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK7-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some embodiments, an ALK7-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an ALK1-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one TGFRII-Fc fusion protein. In some embodiments, an ALK2-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an ALK3-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK4-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an ALK4-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK5-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an ALK5-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK6-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an ALK6-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily

ligands such as those described herein. In some embodiments, an ALK6-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK7-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an ALK7-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ALK1-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ALK2-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ALK3-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK4-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ALK4-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-

Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK5-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ALK5-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK6-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ALK6-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK7-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ALK7-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK7-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one ALK2-Fc fusion protein. In some embodiments, an ALK1-Fc:ALK2-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK2-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK2-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one ALK3-Fc fusion protein. In some embodiments, an ALK1-Fc:ALK3-Fc heteromultimers binds to one or more TGF-beta

superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK3-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK3-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one ALK4-Fc fusion protein. In some embodiments, an ALK1-Fc:ALK4-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK4-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK4-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one ALK5-Fc fusion protein. In some embodiments, an ALK1-Fc:ALK5-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK5-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK5-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one ALK6-Fc fusion protein. In some embodiments, an ALK1-Fc:ALK6-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK6-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK6-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK1-Fc fusion protein and at least one ALK7-Fc fusion protein. In some embodiments, an ALK1-Fc:ALK7-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK7-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK1-Fc:ALK7-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one ALK3-Fc fusion protein. In some

embodiments, an ALK2-Fc:ALK3-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK3-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK3-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one ALK4-Fc fusion protein. In some embodiments, an ALK2-Fc:ALK4-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK4-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK4-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one ALK5-Fc fusion protein. In some embodiments, an ALK2-Fc:ALK5-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK5-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK5-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one ALK6-Fc fusion protein. In some embodiments, an ALK2-Fc:ALK6-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK6-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK6-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK2-Fc fusion protein and at least one ALK7-Fc fusion protein. In some embodiments, an ALK2-Fc:ALK7-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK7-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK2-Fc:ALK7-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one ALK4-Fc fusion protein. In some embodiments, an ALK3-Fc:ALK4-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ALK4-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ALK4-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one ALK5-Fc fusion protein. In some embodiments, an ALK3-Fc:ALK5-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ALK5-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ALK5-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one ALK6-Fc fusion protein. In some embodiments, an ALK3-Fc:ALK6-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ALK6-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ALK6-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK3-Fc fusion protein and at least one ALK7-Fc fusion protein. In some embodiments, an ALK3-Fc:ALK7-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ALK7-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK3-Fc:ALK7-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK4-Fc fusion protein and at least one ALK5-Fc fusion protein. In some embodiments, an ALK4-Fc:ALK5-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ALK5-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands

such as those described herein. In some embodiments, an ALK4-Fc:ALK5-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK4-Fc fusion protein and at least one ALK6-Fc fusion protein. In some embodiments, an ALK4-Fc:ALK6-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ALK6-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ALK6-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK4-Fc fusion protein and at least one ALK7-Fc fusion protein. In some embodiments, an ALK4-Fc:ALK7-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ALK7-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK4-Fc:ALK7-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK5-Fc fusion protein and at least one ALK6-Fc fusion protein. In some embodiments, an ALK5-Fc:ALK6-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:ALK6-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:ALK6-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK5-Fc fusion protein and at least one ALK7-Fc fusion protein. In some embodiments, an ALK5-Fc:ALK7-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:ALK7-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK5-Fc:ALK7-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ALK6-Fc fusion protein and at least one ALK7-Fc fusion protein. In some embodiments, an ALK6-Fc:ALK7-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-

Fc:ALK7-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ALK6-Fc:ALK7-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ActRIIA-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some embodiments, an ActRIIA-Fc:ActRIIB-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIA-Fc:ActRIIB-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIA-Fc:ActRIIB-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ActRIIA-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some embodiments, an ActRIIA-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIA-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIA-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ActRIIA-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an ActRIIA-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIA-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIA-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ActRIIA-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ActRIIA-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIA-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIA-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ActRIIB-Fc fusion protein and at least one BMPRII-Fc fusion protein. In some embodiments, an ActRIIB-Fc:BMPRII-Fc heteromultimers binds to one or more TGF-beta

superfamily ligands such as those described herein. In some embodiments, an ActRIIB-Fc:BMPRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIB-Fc:BMPRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ActRIIB-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an ActRIIB-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIB-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIB-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one ActRIIB-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an ActRIIB-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIB-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an ActRIIB-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one BMPRII-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some embodiments, an BMPRII-Fc:TGFBRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an BMPRII-Fc:TGFBRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an BMPRII-Fc:TGFBRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one BMPRII-Fc fusion protein and at least one MISRII-Fc fusion protein. In some embodiments, an BMPRII-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an BMPRII-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an BMPRII-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects embodiments, the disclosure relates to a heteromultimer comprising at least one TGFBRII-Fc fusion protein and at least one TGFBRII-Fc fusion protein. In some

embodiments, an TGFBRII-Fc:MISRII-Fc heteromultimers binds to one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an TGFBRII-Fc:MISRII-Fc heteromultimers inhibit signaling of one or more TGF-beta superfamily ligands such as those described herein. In some embodiments, an TGFBRII-Fc:MISRII-Fc heteromultimers is a heterodimer.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK1-Fc fusion protein. In some embodiments, the ALK1-Fc fusion protein comprises an ALK1 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids of 22-34 (e.g., amino acid residues 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, and 34) SEQ ID NO: 14, ends at any one of amino acids 95-118 (e.g., amino acid residues 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, and 118) of SEQ ID NO: 14. In some embodiments, the ALK1-Fc fusion protein comprises an ALK1 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22-118 of SEQ ID NO: 14. In some embodiments, the ALK1-Fc fusion protein comprises an ALK1 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-95 of SEQ ID NO: 14. In some embodiments, the ALK1-Fc fusion protein comprises an ALK1 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 14, 15, 124, 126, 413, and 414.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK2-Fc fusion protein. In some embodiments, the ALK2-Fc fusion protein comprises an ALK2 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 21-35 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, and 35) SEQ ID NO: 18, and ends at any one of amino acids 99-123 (e.g., amino acid residues 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, and 123) of SEQ ID NO: 18. In some embodiments, the ALK2-Fc fusion protein comprises an ALK2 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 35-99 of SEQ ID NO: 18. In some embodiments, the ALK2-Fc fusion protein comprises an ALK2 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-123 of SEQ ID NO: 18. In some embodiments, the ALK2-Fc fusion protein comprises an ALK2 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID Nos: 18, 19, 136, 138, 421, and 422.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK3-Fc fusion protein. In some embodiments, the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 24-61 (e.g., amino acid residues 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, and 61) SEQ ID NO: 22, and ends at any one of amino acids 130-152 (e.g., amino acid residues 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, and 152) of SEQ ID NO: 22. In some embodiments, the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 61-130 of SEQ ID NO: 22. In some embodiments, the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-152 of SEQ ID NO: 22. In some embodiments, the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 22, 23, 115, 117, 407, and 408.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK4-Fc fusion protein. In some embodiments, the ALK4-Fc fusion protein comprises an ALK4 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 23-34 (e.g., amino acid residues

23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34) SEQ ID NO: 26 or 83, and ends at any one of amino acids 101-126 (e.g., amino acid residues 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, and 126) of SEQ ID NO: 26 or 83. In some embodiments, the ALK4-Fc fusion protein comprises an ALK4 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-101 of SEQ ID NOs: 26 or 83. In some embodiments, the ALK4-Fc fusion protein comprises an ALK4 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-126 of SEQ ID Nos: 26 or 83. In some embodiments, the ALK4-Fc fusion protein comprises an ALK4 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 403, and 404.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK5-Fc fusion protein. In some embodiments, the ALK5-Fc fusion protein comprises an ALK5 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 25-36 (e.g., amino acid residues 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36) SEQ ID NO: 30 or 87, and ends at any one of amino acids 106-126 (e.g., amino acid residues 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, and 126) of SEQ ID NO: 30 or 87. In some embodiments, the ALK5-Fc fusion protein comprises an ALK5 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 36-106 of SEQ ID NOs: 30 or 87. In some embodiments, the ALK5-Fc fusion protein comprises an ALK5 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-126 of SEQ ID NOs: 30 or 87. In some embodiments, the ALK5-Fc fusion protein comprises an ALK5 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 423, and 424.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK6-Fc fusion protein. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 14-32 (e.g., amino acid residues 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, and 32) SEQ ID NO: 34, and ends at any one of amino acids 102-126 (e.g., amino acid residues 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, and 126) of SEQ ID NO: 34. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 32-102 of SEQ ID NO: 34. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 14-126 of SEQ ID NO: 34. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 425, and 426. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 26-62 (e.g., amino acid residues 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62) SEQ ID NO: 91, and ends at any one of amino acids 132-156 (e.g., amino acid residues 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, and 156) of SEQ ID NO: 91. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 62-132 of SEQ ID NO: 91. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 26-156 of SEQ ID NO: 91.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK7-Fc fusion protein. In some embodiments, the ALK7-Fc fusion protein comprises an ALK7 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 21-28 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, and 28) SEQ ID NO: 38, 305, or 309, and ends at any one of amino acids 92-113 (e.g., amino acid residues 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, and 113) of SEQ ID NO: 38, 305, or 309. In some embodiments, the ALK7-Fc fusion protein comprises an ALK7 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NOs: 38, 305, or 309. In some embodiments, the ALK7-Fc fusion protein comprises an ALK7 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-113 of SEQ ID NOs: 38, 305, or 309. In some embodiments, the ALK7-Fc fusion protein comprises an ALK7 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 405, and 406.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ActRIIA-Fc fusion protein. In some embodiments, the ActRIIA-Fc fusion protein comprises an ActRIIA domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 21-30 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) SEQ ID NO: 9, and ends at any one of amino acids 110-135 (e.g., 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134 or 135) of SEQ ID NO: 9. In some embodiments, the ActRIIA-Fc fusion protein comprises an ActRIIA domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9. In some embodiments, the ActRIIA-Fc fusion protein comprises an ActRIIA domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 9. In some embodiments, the

ActRIIA-Fc fusion protein comprises an ActRIIA domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 9, 10, 11, 118, 120, 409, and 410.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ActRIIB-Fc fusion protein. In some embodiments, the ActRIIB-Fc fusion protein comprises an ActRIIB domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 20-29 (e.g., amino acid residues 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) SEQ ID NO: 1, and ends at any one of amino acids 109-134 (e.g., amino acid residues 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134 of SEQ ID NO: 1. In some embodiments, the ActRIIB-Fc fusion protein comprises an ActRIIB domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, the ActRIIB-Fc fusion protein comprises an ActRIIB domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 20-134 of SEQ ID NO: 1. In some embodiments, the ActRIIB-Fc fusion protein comprises an ActRIIB domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 401, and 402.

In certain aspects, the disclosure relates to a heteromultimer that comprises an BMPRII-Fc fusion protein. In some embodiments, the BMPRII-Fc fusion protein comprises an BMPRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 27-34 (e.g., amino acid residues 27, 28, 29, 30, 31, 32, 33, and 34) SEQ ID NO: 46 or 71, and ends at any one of amino acids 123-150 (e.g., amino acid residues 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150) of SEQ ID NO: 46 or 71. In some embodiments, the BMPRII-Fc fusion protein comprises an BMPRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%,

or 100% identical to amino acids 34-123 of SEQ ID NO: 46 or 71. In some embodiments, the BMPRII-Fc fusion protein comprises an BMPRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 46 or 71. In some embodiments, the BMPRII-Fc fusion protein comprises an BMPRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 411, and 412.

In certain aspects, the disclosure relates to a heteromultimer that comprises an TGFBII-Fc fusion protein. In some embodiments, the TGFBII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and ends at any one of amino acids 168-191 (e.g., 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190 or 191) of SEQ ID NO: 67. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-168 of SEQ ID NO: 67. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 67. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 415, 416, 417, and 418. In some embodiments, the TGFBII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 23-51 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, and 51) of SEQ ID NO: 42, and ends at any one of amino acids 143-166 (e.g., 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, and

166) of SEQ ID NO: 42. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 42. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 42.

In certain aspects, the disclosure relates to a heteromultimer that comprises an MISRII-Fc fusion protein. In some embodiments, the MISRII-Fc fusion protein comprises an MISRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 17-24 (e.g., amino acid residues 17, 18, 19, 20, 21, 22, 23, and 24) SEQ ID NO: 50, 75, or 79, and ends at any one of amino acids 116-149 (e.g., amino acid residues 116, 117, 118, 119, 120, 121, 122 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, and 149) of SEQ ID NO: 50, 75, or 79. In some embodiments, the MISRII-Fc fusion protein comprises an MISRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 50, 75, or 79. In some embodiments, the MISRII-Fc fusion protein comprises an MISRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 50, 75, or 79. In some embodiments, the MISRII-Fc fusion protein comprises an MISRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 50, 51, 75, 76, 79, and 80.

In some embodiments, the TGF-beta superfamily type I and/or type II receptor polypeptides disclosed herein comprise one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent. In some embodiments, the TGF-beta superfamily type I and/or type II polypeptides described herein are glycosylated

and have a glycosylation pattern obtainable from the expression of the polypeptides in a mammalian cell, including, for example, a CHO cell.

In certain aspects the disclosure provides nucleic acids encoding any of the TGF-beta superfamily type I and/or type II polypeptides described herein. Nucleic acids disclosed herein may be operably linked to a promoter for expression, and the disclosure further provides cells transformed with such recombinant polynucleotides. In some embodiments the cell is a mammalian cell such as a COS cell or a CHO cell.

In certain aspects, the disclosure provides methods for making any of the TGF-beta superfamily type I and/or type II polypeptides described herein as well as protein complexes comprising such polypeptides. Such a method may include expressing any of the nucleic acids disclosed herein in a suitable cell (*e.g.*, CHO cell or a COS cell). Such a method may comprise: a) culturing a cell under conditions suitable for expression of the TGF-beta superfamily type I or type II polypeptides described herein, wherein said cell is transformed with a type I or type II polypeptide expression construct; and b) recovering the type I or type II polypeptides so expressed. TGF-beta superfamily type I and/or type II polypeptides described herein, as well as protein complexes of the same, may be recovered as crude, partially purified, or highly purified fractions using any of the well-known techniques for obtaining protein from cell cultures.

In certain aspects, the disclosure provides methods for making any of the heteromultimeric complexes disclosed herein. Such a method may include expressing any of the nucleic acids disclosed herein in a suitable cell (*e.g.*, CHO cell or a COS cell). Such a method may comprise: a) obtaining a cell that comprises a nucleic acid comprising the coding sequence for a TGF-beta superfamily type I receptor polypeptide disclosed herein and a nucleic acid comprising the coding sequence for a TGF-beta superfamily type II receptor polypeptide disclosed herein; (b) culturing such cell under conditions suitable for expression of the TGF-beta superfamily type I and type II polypeptides described herein; and c) recovering the heteromeric complex comprising such type I and type II polypeptides so expressed. Heteromultimeric complexes disclosed herein as crude, partially purified, or highly purified fractions using any of the well-known techniques for obtaining protein from cell cultures.

Any of the protein complexes described herein may be incorporated into a pharmaceutical preparation. Optionally, such pharmaceutical preparations are at least 80%, 85%, 90%, 95%, 97%, 98% or 99% pure with respect to other polypeptide components.

Optionally, pharmaceutical preparations disclosed herein may comprise one or more additional active agents. In some embodiments, heteromultimers of the disclosure comprise less than 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% type I receptor polypeptide homomultimers. In some embodiments, heteromultimers of the disclosure comprise less than 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% type II receptor polypeptide homomultimers. In some embodiments, heteromultimers of the disclosure comprise less than 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% type I receptor polypeptide homomultimers and less than 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% type II receptor polypeptide homomultimers.

The disclosure further provides methods and heteromultimers for use in the treatment or prevention of various disease and disorders associated with, for example, muscle, bone, fat, red blood cells, and other tissues that are affected by one or more ligands of the TGF-beta superfamily. Such disease and disorders include, but are not limited to, disorders associated with muscle loss or insufficient muscle growth (*e.g.*, muscle atrophy; muscular dystrophy, including Duchenne muscular dystrophy, Becker muscular dystrophy, and facioscapulohumeral muscular dystrophy; amyotrophic lateral sclerosis; and cachexia) and disorders associated with undesirable weight gain (*e.g.*, obesity, type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM), cardiovascular disease, hypertension, osteoarthritis, stroke, respiratory problems, and gall bladder disease). In some embodiments, heteromultimeric complexes disclosed herein may be used to decrease body fat content or reduce the rate of increase in body fat content in a subject in need thereof. In some embodiments, heteromultimeric complexes disclosed herein may be used to reduce cholesterol and/or triglyceride levels in a patient.

In some embodiments, heteromeric complexes disclosed herein may be used to treat anemia. In some embodiments, heteromeric complexes disclosed herein may be used to treat thalassemia. In some embodiments, heteromeric complexes disclosed herein may be used to treat myelodysplastinc syndrome. In some embodiments, heteromeric complexes disclosed herein may be used to treat myelofibrosis. In some embodiments, heteromeric complexes disclosed herein may be used to treat a hemoglobinopathy. In some embodiments, heteromeric complexes disclosed herein may be used to treat sickle cell disease. In some embodiments, heteromeric complexes disclosed herein may be used to reduce transfusion burden in a patient in need thereof. In some embodiments, heteromeric complexes disclosed herein may be used to treat a patient with endogenously high erythropoietin levels relative to

the erythropoietin levels of one or more healthy patients of similar age and sex. In some embodiments, heteromeric complexes disclosed herein may be used to treat a patient that has anemia and is non-responsive or intolerate to treatment with EPO (or derivative thereof or an EPO receptor agonist).

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show two schematic examples of heteromeric protein complexes comprising type I receptor and type II receptor polypeptides. Figure 1A depicts a heterodimeric protein complex comprising one type I receptor fusion polypeptide and one type II receptor fusion polypeptide, which can be assembled covalently or noncovalently via a multimerization domain contained within each polypeptide chain. Two assembled multimerization domains constitute an interaction pair, which can be either guided or unguided. Figure 1B depicts a heterotetrameric protein complex comprising two heterodimeric complexes as in Figure 1A. Complexes of higher order can be envisioned.

Figure 2 shows a schematic example of a heteromeric protein complex comprising a type I receptor polypeptide (indicated as "I") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6 or ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 14, 15, 124, 126, 413, 414, 18, 19, 136, 138, 421, 422, 22, 23, 115, 117, 407, 408, 26, 27, 83, 84, 104, 106, 403, 404, 30, 31, 87, 88, 139, 141, 423, 424, 34, 35, 91, 92, 142, 144, 425, 426, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 405, and 406) and a type II receptor polypeptide (indicated as "II") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIA, ActRIIB, MISRII, BMPRII, or TGFBRII protein from humans or other species such as those described herein, e.g., 9, 10, 11, 118, 120, 409, 410, 1, 2, 3, 4, 5, 6, 100, 102, 401, 402, 46, 47, 71, 72, 121, 123, 411, 412, 50, 51, 75, 76, 79, 80, 42, 43, 67, 68, 127, 129, 130, 132, 415, 416, 417, and 418). In the illustrated embodiment, the type I receptor polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C"), and the type II receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("D"). In each fusion polypeptide, a linker may be positioned between the type I or type II receptor polypeptide and the corresponding member of the interaction pair. The first and second members of the interaction pair (C, D) may be a

guided (asymmetric) pair, meaning that the members of the pair associate preferentially with each other rather than self-associate, or the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and may have the same or different amino acid sequences. Traditional Fc fusion proteins and antibodies are examples of unguided interaction pairs, whereas a variety of engineered Fc domains have been designed as guided (asymmetric) interaction pairs [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106].

Figure 3 shows an alignment of extracellular domains of human ActRIIA (SEQ ID NO: 500) and human ActRIIB (SEQ ID NO: 2) with the residues that are deduced herein, based on composite analysis of multiple ActRIIB and ActRIIA crystal structures, to directly contact ligand indicated with boxes.

Figure 4 shows a multiple sequence alignment of various vertebrate ActRIIB precursor proteins without their intracellular domains (SEQ ID NOs: 501, 502, 503, 504, 505, and 506, respectively), human ActRIIA precursor protein without its intracellular domain (SEQ ID NO: 507), and a consensus ActRII precursor protein (SEQ ID NO: 508).

Figure 5 shows multiple sequence alignment of Fc domains from human IgG isotypes using Clustal 2.1. Hinge regions are indicated by dotted underline. Double underline indicates examples of positions engineered in IgG1 Fc to promote asymmetric chain pairing and the corresponding positions with respect to other isotypes IgG2, IgG3 and IgG4.

Figure 6 shows ligand binding data for an ActRIIB-Fc:ALK4-Fc heterodimeric protein complex as compared to ActRIIB-Fc homodimer and ALK4-Fc homodimer. For each protein complex, ligands are ranked by k_{off}, a kinetic constant that correlates well with ligand signaling inhibition, and listed in descending order of binding affinity (ligands bound most tightly are listed at the top). At left, yellow, red, green, and blue lines indicate magnitude of the off-rate constant. Solid black lines indicate ligands whose binding to heterodimer is enhanced or unchanged compared with homodimer, whereas dashed red lines indicate substantially reduced binding compared with homodimer. As shown, the ActRIIB-Fc:ALK4-Fc heterodimer displays enhanced binding to activin B compared with either homodimer, retains strong binding to activin A, GDF8, and GDF11 as observed with ActRIIB-Fc homodimer, and exhibits substantially reduced binding to BMP9, BMP10, and GDF3. Like ActRIIB-Fc homodimer, the heterodimer retains intermediate-level binding to BMP6.

Figure 7 shows ligand binding data for an ActRIIB-Fc:ALK3-Fc heterodimeric protein complex as compared to ActRIIB-Fc homodimer and ALK3-Fc homodimer. Format is the same as in Figure 6. As shown, the ActRIIB-Fc:ALK3-Fc heterodimer binds BMP2 and BMP4 with exceptionally high affinity and displays greatly enhanced binding to BMP5, BMP6, BMP7, GDF5, GDF6, and GDF7 compared with either homodimer. Compared to ActRIIB homodimer, the ActRIIB-Fc:ALK3-Fc heterodimer displays reduced binding to activin A, activin B, BMP10, GDF8, and GDF11 and also discriminates among these ligands to a greater degree, particularly between activin A and activin B. In addition, the ability of ActRIIB-Fc homodimer to bind BMP9 and GDF3 with high affinity is absent for ActRIIB-Fc:ALK3-Fc heterodimer.

Figure 8 shows ligand binding data for an ActRIIB-Fc:ALK7-Fc heterodimeric protein complex as compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer. Format is the same as in Figure 6. As shown, four of the five ligands with strong binding to ActRIIB-Fc homodimer (activin A, BMP10, GDF8, and GDF11) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer, the exception being activin B which retains tight binding to the heterodimer. In addition, three ligands with intermediate binding to ActRIIB-Fc homodimer (GDF3, BMP6, and particularly BMP9) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer. In contrast, BMP5 binds the ActRIIB-Fc:ALK7 heterodimer with intermediate strength despite only weak binding to ActRIIB-Fc homodimer. No ligands tested bind to ALK7-Fc homodimer.

Figure 9 shows ligand binding data for an ActRIIB-Fc:ALK2-Fc heterodimeric protein complex as compared to ActRIIB-Fc homodimer and ALK2-Fc homodimer. Format is the same as in Figure 6. As shown, the ActRIIB-Fc:ALK2-Fc heterodimer exhibits preferential and strong binding to activin B, thus resembling ActRIIB-Fc:ALK7-Fc heterodimer (Fig. 8). However, ActRIIB-Fc:ALK2-Fc heterodimer differs from ActRIIB-Fc:ALK7-Fc in part by retaining the tight binding to BMP9 characteristic of ActRIIB-Fc homodimer. No ligands tested bind to ALK2-Fc homodimer.

Figure 10 shows ligand binding data for an ActRIIA-Fc:ALK4-Fc heterodimeric protein complex as compared to ActRIIA-Fc homodimer and ALK4-Fc homodimer. Format is the same as in Figure 6. As shown, the ActRIIA-Fc:ALK4-Fc heterodimer exhibits enhanced binding to activin A, and particularly enhanced binding to activin AC, compared to ActRIIA-Fc homodimer, while retaining strong binding to activin AB and GDF11. In

addition, the ligand with highest affinity for ActRIIA-Fc homodimer, activin B, displays reduced affinity (albeit still within the high-affinity range) for the ActRIIA-Fc:ALK4-Fc heterodimer. The ActRIIA-Fc:ALK4-Fc heterodimer also exhibits markedly reduced binding to BMP10 compared to ActRIIA-Fc homodimer.

Figure 11 shows ligand binding data for a BMPRII-Fc:ALK1-Fc heterodimeric protein complex as compared to ActRIIB-Fc homodimer and ALK1-Fc homodimer. Format is the same as in Figure 6. As shown, the BMPRII-Fc:ALK1-Fc heterodimer largely retains the strong binding to BMP9 and BMP10 characteristic of ALK1-Fc homodimer; however, the heterodimer displays modest selectivity for BMP10 over BMP9 not present with the homodimer. Also unlike ALK1-Fc homodimer, the BMPRII-Fc:ALK1-Fc heterodimer binds to BMP15, albeit with an off-rate approximately ten times faster than that of BMPRII-Fc homodimer.

Figure 12 shows ligand binding data for a BMPRII-Fc:ALK3-Fc heterodimeric protein complex as compared to BMPRII-Fc homodimer and ALK3-Fc homodimer. Format is the same as in Figure 6. As shown, the BMPRII-Fc:ALK3-Fc heterodimer binds much more strongly to BMP6 than does ALK3-Fc homodimer, reflecting an off-rate nearly ten times slower. With its largely unchanged binding to BMP2 and BMP4, the BMPRII-Fc:ALK3 heterodimer can therefore be considered a joint inhibitor of BMP2, BMP4, and BMP6. This binding profile contrasts with that of ALK3-Fc homodimer, whose exceptionally strongly binding to BMP4 and BMP2 identifies it as highly selective for this ligand pair compared to four ligands with intermediate-level binding, including BMP6.

Figure 13 shows ligand binding data for a BMPRII-Fc:ALK4-Fc heterodimeric protein complex as compared to BMPRII-Fc homodimer and ALK4-Fc homodimer. Format is the same as in Figure 6. BMPRII-Fc:ALK4-Fc heterodimer differs from both homodimers by binding several activin ligands with high or intermediate strength and differs from BMPRII-Fc homodimer by binding BMP15 only weakly. Most notably, BMPRII-Fc:ALK4-Fc heterodimer binds strongly and with high selectivity to the heterodimeric ligand activin AB.

Figure 14 shows ligand binding data for two different TGFβRII-Fc:ALK5-Fc heterodimeric protein complexes as compared to TGFβRII-Fc homodimer and ALK5-Fc homodimer. Format is the same as in Figure 6. As shown, TGFβRII-Fc:ALK5-Fc heterodimers differ markedly from TGFβRII-Fc homodimer in their high selectivity for

TGFβ2 while still retaining considerable affinity for TGFβ1 and TGFβ3. The heterodimer incorporating the long isoform of TGFβRII bound TGFβ2 more strongly and selectively than did its short-isoform counterpart. No ligands tested bind to ALK5-Fc homodimer.

Figures 15A-15D show schematic examples of heteromeric protein complexes comprising a type I receptor polypeptide (indicated as "I") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6 or ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 14, 15, 124, 126, 413, 414, 18, 19, 136, 138, 421, 422, 22, 23, 115, 117, 407, 408, 26, 27, 83, 84, 104, 106, 403, 404, 30, 31, 87, 88, 139, 141, 423, 424, 34, 35, 91, 92, 142, 144, 425, 426, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 405, and 406) and a type II receptor polypeptide (indicated as "II") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIA, ActRIIB, MISRII, BMPRII, or TGFBRII protein from humans or other species such as those described herein, e.g., 9, 10, 11, 118, 120, 409, 410, 1, 2, 3, 4, 5, 6, 100, 102, 401, 402, 46, 47, 71, 72, 121, 123, 411, 412, 50, 51, 75, 76, 79, 80, 42, 43, 67, 68, 127, 129, 130, 132, 415, 416, 417, and 418). In the illustrated embodiments, the a type I receptor polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"), and a type II receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"). Suitable interaction pairs included, for example, heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof such as those described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. In each fusion polypeptide, a linker may be positioned between the a type I receptor polypeptide or a type II receptor polypeptide and the corresponding member of the interaction pair. The first and second members of the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference, and they may have the same or different amino acid sequences. See Figure 15A. Alternatively, the interaction pair may be a guided (asymmetric) pair, meaning that the members of the pair associate preferentially with each other rather than self-associate. See Figure 15B. Complexes of higher order can be envisioned. See Figure 15C and 15D.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

In part, the present disclosure relates to heteromultimers comprising an extracellular domain of a TGFβ superfamily type I receptor polypeptide and an extracellular domain of a TGFβ superfamily type II receptor polypeptide, heteromultimers comprising an extracellular domain of at least two different TGFβ superfamily type I receptor polypeptides, heteromultimers comprising an extracellular domain of at least two different TGFβ superfamily type II receptor polypeptides, methods of making such heteromultimers, and uses thereof. As described herein, in some embodiments, heteromultimers may comprise an extracellular domain of a TGFβ superfamily type I receptor polypeptide selected from: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7. Similarly, in some embodiments, these heteromultimers may comprise an extracellular domain of a TGFβ superfamily type II receptor polypeptide selected from: ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII. In certain preferred embodiments, heteromultimers of the disclosure have an altered TGFβ superfamily ligand binding specificity/profile relative to a corresponding sample of a homomultimer (*e.g.*, an ActRIIB:ALK4 heterodimer compared to an ActRIIB:ActRIIB homodimer or an ALK4:ALK4 homodimer).

The TGF-β superfamily is comprised of over 30 secreted factors including TGF-betas, activins, nodals, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), and anti-Mullerian hormone (AMH). See, *e.g.*, Weiss *et al.* (2013) Developmental Biology, 2(1): 47-63. Members of the superfamily, which are found in both vertebrates and invertebrates, are ubiquitously expressed in diverse tissues and function during the earliest stages of development throughout the lifetime of an animal. Indeed, TGF-β superfamily proteins are key mediators of stem cell self-renewal, gastrulation, differentiation, organ morphogenesis, and adult tissue homeostasis. Consistent with this ubiquitous activity, aberrant TGF-beta superfamily signaling is associated with a wide range of human pathologies including, for example, autoimmune disease, cardiovascular disease, fibrotic disease, and cancer.

Ligands of the TGF-beta superfamily share the same dimeric structure in which the central 3-1/2 turn helix of one monomer packs against the concave surface formed by the beta-strands of the other monomer. The majority of TGF-beta family members are further stabilized by an intermolecular disulfide bonds. This disulfide bond traverses through a ring formed by two other disulfide bonds generating what has been termed a 'cysteine knot' motif.

See, *e.g.*, Lin et al., (2006) Reproduction 132: 179-190 and Hinck et al. (2012) FEBS Letters 586: 1860-1870.

TGF-beta superfamily signaling is mediated by heteromeric complexes of type I and type II serine/threonine kinase receptors, which phosphorylate and activate downstream SMAD proteins (*e.g.*, SMAD proteins 1, 2, 3, 5, and 8) upon ligand stimulation. See, *e.g.*, Massagué (2000) Nat. Rev. Mol. Cell Biol. 1:169-178. These type I and type II receptors are transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase specificity. In general, type I receptors mediate intracellular signaling while the type II receptors are required for binding TGF-beta superfamily ligands. Type I and II receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors.

The TGF-beta family can be divided into two phylogenetic branches based on the type I receptors they bind and the Smad proteins they activate. One is the more recently evolved branch, which includes, *e.g.*, the TGF-betas, activins, GDF8, GDF9, GDF11, BMP3 and nodal, which signal through type I receptors that activate Smads 2 and 3 [Hinck (2012) FEBS Letters 586:1860-1870]. The other branch comprises the more distantly related proteins of the superfamily and includes, *e.g.*, BMP2, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF1, GDF5, GDF6, and GDF7, which signal through Smads 1, 5, and 8.

TGF-beta isoforms are the founding members of the TGF-beta superfamily, of which there are 3 known isoforms in mammals designated as TGF-beta1, TGF-beta2 and TGF-beta3. Mature bioactive TGF-beta ligands function as homodimers and predominantly signal through the type I receptor ALK5, but have also been found to additionally signal through ALK1 in endothelial cells. See, *e.g.*, Goumans *et al.* (2003) Mol Cell 12(4): 817-828. TGF-beta1 is the most abundant and ubiquitously expressed isoform. TGF-beta1 is known to have an important role in wound healing, and mice expressing a constitutively active TGF-beta1 transgene develop fibrosis. See *e.g.*, Clouthier *et al.*, (1997) J Clin. Invest. 100(11): 2697-2713. TGF-beta1 is also involved in T cell activation and maintenance of T regulatory cells. See, *e.g.*, Li *et al.*, (2006) Immunity 25(3): 455-471. TGF-beta2 expression was first described in human glioblastoma cells, and is occurs in neurons and astroglial cells of the embryonic nervous system. TGF-beta2 is known to suppress interleukin-2-dependent growth of T lymphocytes. TGF-beta3 was initially isolated from a human rhabdomyosarcoma cell

line and since has been found in lung adenocarcinoma and kidney carcinoma cell lines. TGF-beta3 is known to be important for palate and lung morphogenesis. See, *e.g.*, Kubiczkova et al., (2012) Journal of Translational Medicine 10:183.

Activins are members of the TGF-beta superfamily and were initially discovered as regulators of secretion of follicle-stimulating hormone, but subsequently various reproductive and non-reproductive roles have been characterized. There are three principal activin forms (A, B, and AB) that are homo/heterodimers of two closely related β subunits ($\beta_A\beta_A$, $\beta_B\beta_B$, and $\beta_A\beta_B$, respectively). The human genome also encodes an activin C and an activin E, which are primarily expressed in the liver, and heterodimeric forms containing β_C or β_E are also known. In the TGF-beta superfamily, activins are unique and multifunctional factors that can stimulate hormone production in ovarian and placental cells, support neuronal cell survival, influence cell-cycle progress positively or negatively depending on cell type, and induce mesodermal differentiation at least in amphibian embryos. See, *e.g.*, DePaolo *et al.* (1991) Proc Soc Ep Biol Med. 198:500-512; Dyson *et al.* (1997) Curr Biol. 7:81-84; and Woodruff (1998) Biochem Pharmacol. 55:953-963. In several tissues, activin signaling is antagonized by its related heterodimer, inhibin. For example, in the regulation of follicle-stimulating hormone (FSH) secretion from the pituitary, activin promotes FSH synthesis and secretion, while inhibin reduces FSH synthesis and secretion. Other proteins that may regulate activin bioactivity and/or bind to activin include follistatin (FS), follistatin-related protein (FSRP, also known as FLRG or FSTL3), and α_2-macroglobulin.

As described herein, agents that bind to "activin A" are agents that specifically bind to the β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (*e.g.*, a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of a heterodimer complex (*e.g.*, a $\beta_A\beta_B$ heterodimer), agents that bind to "activin A" are specific for epitopes present within the β_A subunit, but do not bind to epitopes present within the non-β_A subunit of the complex (*e.g.*, the β_B subunit of the complex). Similarly, agents disclosed herein that antagonize (inhibit) "activin A" are agents that inhibit one or more activities as mediated by a β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (*e.g.*, a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of $\beta_A\beta_B$ heterodimers, agents that inhibit "activin A" are agents that specifically inhibit one or more activities of the β_A subunit, but do not inhibit the activity of the non-β_A subunit of the complex (*e.g.*, the β_B subunit of the complex). This principle applies also to agents that *bind to* and/or *inhibit* "activin B", "activin C", and "activin E". Agents disclosed herein that antagonize "activin AB", "activin AC", "activin

AE", "activin BC", or "activin BE" are agents that inhibit one or more activities as mediated by the β_A subunit and one or more activities as mediated by the β_B subunit. The same principle applies to agents that bind to and/or inhibit "activin AC", "activin AE", "activin BC", or "activin BE".

Nodal proteins have functions in mesoderm and endoderm induction and formation, as well as subsequent organization of axial structures such as heart and stomach in early embryogenesis. It has been demonstrated that dorsal tissue in a developing vertebrate embryo contributes predominantly to the axial structures of the notochord and pre-chordal plate while it recruits surrounding cells to form non-axial embryonic structures. Nodal appears to signal through both type I and type II receptors and intracellular effectors known as SMAD proteins. Studies support the idea that ActRIIA and ActRIIB serve as type II receptors for nodal. See, *e.g.*, Sakuma *et al.* (2002) Genes Cells. 2002, 7:401-12. It is suggested that Nodal ligands interact with their co-factors (*e.g.*, Cripto or Cryptic) to activate activin type I and type II receptors, which phosphorylate SMAD2. Nodal proteins are implicated in many events critical to the early vertebrate embryo, including mesoderm formation, anterior patterning, and left-right axis specification. Experimental evidence has demonstrated that nodal signaling activates pAR3-Lux, a luciferase reporter previously shown to respond specifically to activin and TGF-beta. However, nodal is unable to induce pTlx2-Lux, a reporter specifically responsive to bone morphogenetic proteins. Recent results provide direct biochemical evidence that nodal signaling is mediated by SMAD2 and SMAD3, which also mediate signaling by TGF-betas and activins. Further evidence has shown that the extracellular protein Cripto or Cryptic is required for nodal signaling, making it distinct from activin or TGF-beta signaling.

The BMPs and GDFs together form a family of cysteine-knot cytokines sharing the characteristic fold of the TGF-beta superfamily. See, *e.g.*, Rider *et al.* (2010) Biochem J., 429(1):1-12. This family includes, for example, BMP2, BMP4, BMP6, BMP7, BMP2a, BMP3, BMP3b (also known as GDF10), BMP4, BMP5, BMP6, BMP7, BMP8, BMP8a, BMP8b, BMP9 (also known as GDF2), BMP10, BMP11 (also known as GDF11), BMP12 (also known as GDF7), BMP13 (also known as GDF6), BMP14 (also known as GDF5), BMP15, GDF1, GDF3 (also known as VGR2), GDF8 (also known as myostatin), GDF9, GDF15, and decapentaplegic. Besides the ability to induce bone formation, which gave the BMPs their name, the BMP/GDFs display morphogenetic activities in the development of a wide range of tissues. BMP/GDF homo- and hetero-dimers interact with combinations of

type I and type II receptor dimers to produce multiple possible signaling complexes, leading to the activation of one of two competing sets of SMAD transcription factors. BMP/GDFs have highly specific and localized functions. These are regulated in a number of ways, including the developmental restriction of BMP/GDF expression and through the secretion of several specific BMP antagonist proteins that bind with high affinity to the cytokines. Curiously, a number of these antagonists resemble TGF-beta superfamily ligands.

Growth and differentiation factor-8 (GDF8) is also known as myostatin. GDF8 is a negative regulator of skeletal muscle mass and is highly expressed in developing and adult skeletal muscle. The GDF8 null mutation in transgenic mice is characterized by a marked hypertrophy and hyperplasia of skeletal muscle. See, *e.g.*, McPherron *et al.*, Nature (1997) 387:83-90. Similar increases in skeletal muscle mass are evident in naturally occurring mutations of GDF8 in cattle and, strikingly, in humans. *See*, *e.g.*, Ashmore *et al.* (1974) Growth, 38:501-507; Swatland and Kieffer, J. Anim. Sci. (1994) 38:752-757; McPherron and Lee, Proc. Natl. Acad. Sci. USA (1997) 94:12457-12461; Kambadur *et al.*, Genome Res. (1997) 7:910-915; and Schuelke *et al.* (2004) N Engl J Med, 350:2682-8. Studies have also shown that muscle wasting associated with HIV-infection in humans is accompanied by increases in GDF8 protein expression. See, *e.g.*, Gonzalez-Cadavid *et al.*, PNAS (1998) 95:14938-43. In addition, GDF8 can modulate the production of muscle-specific enzymes (*e.g.*, creatine kinase) and modulate myoblast cell proliferation. See, *e.g.*, International Patent Application Publication No. WO 00/43781). The GDF8 propeptide can noncovalently bind to the mature GDF8 domain dimer, inactivating its biological activity. See, *e.g.*, Miyazono *et al.* (1988) J. Biol. Chem., 263: 6407-6415; Wakefield *et al.* (1988) J. Biol. Chem., 263; 7646-7654; and Brown *et al.* (1990) Growth Factors, 3: 35-43. Other proteins which bind to GDF8 or structurally related proteins and inhibit their biological activity include follistatin, and potentially, follistatin-related proteins. See, *e.g.*, Gamer *et al.* (1999) Dev. Biol., 208: 222-232.

GDF11, also known as BMP11, is a secreted protein that is expressed in the tail bud, limb bud, maxillary and mandibular arches, and dorsal root ganglia during mouse development. See, *e.g.*, McPherron *et al.* (1999) Nat. Genet., 22: 260-264; and Nakashima *et al.* (1999) Mech. Dev., 80: 185-189. GDF11 plays a unique role in patterning both mesodermal and neural tissues. See, *e.g.*, Gamer *et al.* (1999) Dev Biol., 208:222-32. GDF11 was shown to be a negative regulator of chondrogenesis and myogenesis in developing chick limb. See, *e.g.*, Gamer *et al.* (2001) Dev Biol., 229:407-20. The expression of GDF11 in

muscle also suggests its role in regulating muscle growth in a similar way to GDF8. In addition, the expression of GDF11 in brain suggests that GDF11 may also possess activities that relate to the function of the nervous system. Interestingly, GDF11 was found to inhibit neurogenesis in the olfactory epithelium. See, *e.g.*, Wu *et al.* (2003) Neuron., 37:197-207. Hence, GDF11 may have in vitro and in vivo applications in the treatment of diseases such as muscle diseases and neurodegenerative diseases (*e.g.*, amyotrophic lateral sclerosis).

BMP7, also called osteogenic protein-1 (OP-1), is well known to induce cartilage and bone formation. In addition, BMP7 regulates a wide array of physiological processes. For example, BMP7 may be the osteoinductive factor responsible for the phenomenon of epithelial osteogenesis. It is also found that BMP7 plays a role in calcium regulation and bone homeostasis. Like activin, BMP7 binds to type II receptors, ActRIIA and ActRIIB. However, BMP7 and activin recruit distinct type I receptors into heteromeric receptor complexes. The major BMP7 type I receptor observed was ALK2, while activin bound exclusively to ALK4 (ActRIIB). BMP7 and activin elicited distinct biological responses and activated different SMAD pathways. See, *e.g.*, Macias-Silva *et al.* (1998) J Biol Chem. 273:25628-36.

Anti-Mullerian hormone (AMH), also known as Mullerian-inhibiting substance (MIS), is a TGF-beta family glycoprotein. One AMH-associated type II receptor has been identified and is designated as AMHRII, or alternatively MISRII. AMH induces regression of the Mullerian ducts in the human male embryo. AMH is expressed in reproductive age women and does not fluctuate with cycle or pregnancy, but was found to gradual decrease as both oocyte quantity and quality decrease, suggesting AMH could serve as a biomarker for ovarian physiology. See *e.g.* Zec *et al.*, (2011) Biochemia Medica 21(3): 219-30.

Activin receptor-like kinase-1 (ALK1), the product of the *ACVRL1* gene known alternatively as ACVRLK1, is a type I receptor whose expression is predominantly restricted to endothelial cells. See, *e.g.*, OMIM entry 601284. ALK1 is activated by the binding of TGF-beta family ligands such as BMP9 and BMP10, and ALK1 signaling is critical in the regulation of both developmental and pathological blood vessel formation. ALK1 expression overlaps with sites of vasculogenesis and angiogenesis in early mouse development, and ALK1 knockout mice die around embryonic day 11.5 because of severe vascular abnormalities (see *e.g.*, Cunha and Pietras (2011) Blood 117(26):6999-7006.) ALK1 expression has also been described in other cell types such as hepatic stellate cells and chondrocytes. Additionally, ALK1 along with activin receptor-like kinase-2 (ALK2) have

been found to be important for BMP9-induced osteogenic signaling in mesenchymal stem cells. See *e.g.*, Cunha and Pietras (2011) Blood 117(26):6999-7006.

ALK2, the product of the *ACVR1* gene known alternatively as ActRIA or ACVRLK2, is a type I receptor that has been shown to bind activins and BMPs. ALK2 is critical for embryogenesis as ALK2 knockout mice die soon after gastrulation. See, *e.g.*, Mishina *et al.* (1999) Dev Biol. 213: 314-326 and OMIM entry 102576. Constitutively active mutations in ALK2 are associated with fibrodysplasia ossificans progressiva (FOP). FOP is rare genetic disorder that causes fibrous tissue, including muscle, tendon and ligament, to be ossified spontaneously or when damaged. An arginine to histidine mutation in codon 206 of ALK2 is naturally occurring mutation associated with FOP in humans. This mutation induces BMP-specific signaling via ALK2 without the binding of ligand. See, *e.g.*, Fukuda *et al.*, (2009) J Biol Chem. 284(11):7149-7156 and Kaplan *et al.*, (2011) Ann N.Y. Acad Sci. 1237: 5-10.

Activin receptor-like kinase-3 (ALK3), the product of the *BMPR1A* gene known alternatively as ACVRLK3, is a type I receptor mediating effects of multiple ligands in the BMP family. Unlike several type I receptors with ubiquitous tissue expression, ALK3 displays a restricted pattern of expression consistent with more specialized functionality. See, *e.g.*, ten Dijke (1993) Oncogene, 8: 2879-2887 and OMIM entry 601299. ALK3 is generally recognized as a high affinity receptor for BMP2, BMP4, BMP7 and other members of the BMP family. BMP2 and BMP7 are potent stimulators of osteoblastic differentiation, and are now used clinically to induce bone formation in spine fusions and certain non-union fractures. ALK3 is regarded as a key receptor in mediating BMP2 and BMP4 signaling in osteoblasts. See, *e.g.*, Lavery *et al.* (2008) J. Biol. Chem. 283: 20948-20958. A homozygous ALK3 knockout mouse dies early in embryogenesis (~day 9.5), however, adult mice carrying a conditional disruption of ALK3 in osteoblasts have been recently reported to exhibit increased bone mass, although the newly formed bone showed evidence of disorganization. See, *e.g.*, Kamiya (2008) J. Bone Miner. Res., 23:2007-2017; and Kamiya (2008) Development 135: 3801-3811. This finding is in startling contrast to the effectiveness of BMP2 and BMP7 (ligands for ALK3) as bone building agents in clinical use.

Activin receptor-like kinase-4 (ALK4), the product of the *ACVR1B* gene alternatively known as ACVRLK4, is a type I receptor that transduces signaling for a number of TGF-beta family ligands including activins, nodal and GDFs. ALK4 mutations are associated with pancreatic cancer and expression of dominant negative truncated ALK4 isoforms are highly

expressed in human pituitary tumors. See, *e.g.*, Tsuchida *et al.*, (2008) Endocrine Journal 55(1):11-21 and OMIM entry 601300.

Activin receptor-like kinase-5 (ALK5), the product of the *TGFBR1* gene, is widely expressed in most cell types. Several TGF-beta superfamily ligands, including TGF-betas, activin, and GDF-8, signal via ALK5 and activate downstream Smad 2 and Smad 3. Mice deficient in ALK5 exhibit severe defects in the vascular development of the yolk sac and placenta, lack circulating red blood cells, and die mid-gestation. It was found that these embryos had normal hematopoietic potential, but enhanced proliferation and improper migration of endothelial cells. Thus, ALK5-dependent signaling is important for angiogenesis, but not for the development of hematopoietic progenitor cells and functional hematopoiesis. See, *e.g.* Larsson *et al.*, (2001) The EMBO Journal, 20(7): 1663-1673 and OMIM entry 190181. In endothelial cells, ALK5 acts cooperatively and opposite to ALK1 signaling. ALK5 inhibits cell migration and proliferation, notably the opposite effect of ALK1. See, *e.g.*, Goumans *et al.* (2003) Mol Cell 12(4): 817-828. Additionally, ALK5 is believed to negatively regulate muscle growth. Knockdown of ALK5 in the muscle a mouse model of muscular dystrophy was found to decrease fibrosis and increase expression of genes associate with muscle growth. See, *e.g.* Kemaladewi *et al.*, (2014) Mol Ther Nucleic Acids 3, e156.

Activin receptor-like kinase-6 (ALK6) is the product of the *BMPR1B* gene, whose deficiency is associated with chrondodysplasia and limb defects in both humans and mice. See, *e.g.*, Demirhan *et al.*, (2005) J Med Genet. 42:314-317. ALK6 is widely expressed throughout the developing skeleton, and is required for chondrogenesis in mice. See, *e.g.*, Yi *et al.*, (2000) Development 127:621-630 and OMIM entry 603248.

Activin receptor-like kinase-7 (ALK7) is the product of the *ACVR1C* gene. ALK7 null mice are viable, fertile, and display no skeletal or limb malformations. GDF3 signaling through ALK7 appears to play a role in insulin sensitivity and obesity. This is supported by results that Alk7 null mice show reduced fat accumulation and resistance to diet-induced obesity. See, *e.g.*, Andersson *et al.*, (2008) PNAS 105(20): 7252-7256. ALK7-mediated Nodal signaling has been implicated to have both tumor promoting and tumor suppressing effects in a variety of different cancer cell lines. See, *e.g.*, De Silva *et al.*, (2012) Frontiers in Endocrinology 3:59 and OMIM entry 608981.

As used herein the term "ActRII" refers to the family of type II activin receptors. This family includes both the activin receptor type IIA (ActRIIA), encoded by the *ACVR2A* gene, and the activin receptor type IIB (ActRIIB), encoded by the *ACVR2B* gene. ActRII receptors are TGF-beta superfamily type II receptors that bind a variety of TGF-beta superfamily ligands including activins, GDF8 (myostatin), GDF11, and a subset of BMPs, notably BMP6 and BMP7. ActRII receptors are implicated in a variety of biological disorders including muscle and neuromuscular disorders (e.g., muscular dystrophy, amyotrophic lateral sclerosis (ALS), and muscle atrophy), undesired bone/cartilage growth, adipose tissue disorders (e.g., obesity), metabolic disorders (e.g., type 2 diabetes), and neurodegenerative disorders. See, *e.g.*, Tsuchida *et al.*, (2008) Endocrine Journal 55(1):11-21, Knopf *et al.*, U.S.8,252,900, and OMIM entries 102581 and 602730.

Transforming growth factor beta receptor II (TGFBRII), encoded by the *TGFBR2* gene, is a type II receptor that is known to bind TGF-beta ligands and activate downstream Smad 2 and Smad 3 effectors. See, *e.g.*, Hinck (2012) FEBS Letters 586: 1860-1870 and OMIM entry 190182. TGF-beta signaling through TGFBRII is critical in T-cell proliferation, maintenance of T regulatory cells and proliferation of precartilaginous stem cells. See, *e.g.*, Li *et al.*, (2006) Immunity 25(3): 455-471 and Cheng *et al.*, Int. J. Mol. Sci. 2014, 15, 12665-12676.

Bone morphogenetic protein receptor II (BMPRII), encoded by the *BMPR2* gene, is a type II receptor that is thought to bind certain BMP ligands. In some instances, efficient ligand binding to BMPRII is dependent on the presence of the appropriate TGFBR type I receptors. See, *e.g.*, Rosenzweig *et al.*, (1995) PNAS 92:7632-7636. Mutations in BMPRII are associated pulmonary hypertension in humans. See OMIM entry 600799.

Müllerian-inhibiting substance receptor II (MISRII), the product of the *AMHR2* gene known alternatively as anti-Müllerian hormone type II receptor, is a type II TGF-beta receptor. MISRII binds the MIS ligand, but requires the presence of an appropriate type I receptor, such as ALK3 or ALK6, for signal transduction. See, *e.g.*, Hinck (2012) FEBS Letters 586:1860-1870 and OMIM entry 600956. MISRII is involved in sex differentiation in humans and is required for Müllerian regression in the human male. AMH is expressed in reproductive age women and does not fluctuate with cycle or pregnancy, but was found to gradual decrease as both oocyte quantity and quality decrease, suggesting AMH could serve as a biomarker of ovarian physiology. See, *e.g.*, Zec *et al.*, (2011) Biochemia Medica 21(3): 219-30 and OMIM entry 600956.

In certain aspects, the present disclosure relates to the use of a) heteromultimers comprising an extracellular domain of a TGFβ superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) and an extracellular domain of a TGFβ superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII) b) heteromultimers comprising an extracellular domain of at least two TGFβ superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7), and heteromultimers comprising an extracellular domain of at least two TGFβ superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII), preferably soluble heteromultimers, to antagonize intracellular signaling transduction (*e.*g., Smad 2/3 and/or Smad 1/5/8 signaling) initiated by one or more TGFβ superfamily ligands (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, Müllerian-inhibiting substance (MIS), and Lefty). As described herein, such antagonist heteromultimer complexes may be useful in the treatment or prevention of various disorders/conditions associated with, *e.g.*, muscle loss, insufficient muscle growth, neurodegeneration, bone loss, reduced bone density and/or mineralization, insufficient bone growth, metabolic disorders such as obesity and red blood cell disorders such as anemia.

In particular, the data of the present disclosure demonstrates that heteromultimers comprising an extracellular domain of a TGFβ superfamily type I receptor polypeptide and an extracellular domain of a TGFβ superfamily type II receptor polypeptide have different ligand binding specificities/profiles in comparison to their corresponding homomultimer complexes.

The terms used in this specification generally have their ordinary meanings in the art, within the context of this disclosure and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification to provide additional guidance to the practitioner in describing the compositions and methods of the disclosure and how to make and use them. The scope or meaning of any use of a term will be apparent from the specific context in which it is used.

The terms "heteromer" or "heteromultimer" is a complex comprising at least a first polypeptide and a second polypeptide, wherein the second polypeptide differs in amino acid

sequence from the first polypeptide by at least one amino acid residue. The heteromer can comprise a "heterodimer" formed by the first and second polypeptide or can form higher order structures where polypeptides in addition to the first and second polypeptide are present. Exemplary structures for the heteromultimer include, for example, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. Heterodimers are designated herein as X:Y or equivalently as X-Y, where X represents a first polypeptide and Y represents a second polypeptide. In certain embodiments a heteromultimer is recombinant (e.g., one or more polypeptide components may be a recombinant protein), isolated and/or purified protein complex.

"Homologous," in all its grammatical forms and spelling variations, refers to the relationship between two proteins that possess a "common evolutionary origin," including proteins from superfamilies in the same species of organism, as well as homologous proteins from different species of organism. Such proteins (and their encoding nucleic acids) have sequence homology, as reflected by their sequence similarity, whether in terms of percent identity or by the presence of specific residues or motifs and conserved positions. However, in common usage and in the instant application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and may or may not relate to a common evolutionary origin.

The term "sequence similarity," in all its grammatical forms, refers to the degree of identity or correspondence between nucleic acid or amino acid sequences that may or may not share a common evolutionary origin.

"Percent (%) sequence identity" with respect to a reference polypeptide (or nucleotide) sequence is defined as the percentage of amino acid residues (or nucleic acids) in a candidate sequence that are identical to the amino acid residues (or nucleic acids) in the reference polypeptide (nucleotide) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid (nucleic acid) sequence identity values are generated

using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

"Agonize", in all its grammatical forms, refers to the process of activating a protein and/or gene (*e.g.*, by activating or amplifying that protein's gene expression or by inducing an inactive protein to enter an active state) or increasing a protein's and/or gene's activity.

"Antagonize", in all its grammatical forms, refers to the process of inhibiting a protein and/or gene (e.g., by inhibiting or decreasing that protein's gene expression or by inducing an active protein to enter an inactive state) or decreasing a protein's and/or gene's activity.

The terms "about" and "approximately" as used in connection with a numerical value throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. In general, such interval of accuracy is ± 10%. Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably ≤ 5 -fold and more preferably ≤ 2-fold of a given value.

Numeric ranges disclosed herein are inclusive of the numbers defining the ranges.

The terms "a" and "an" include plural referents unless the context in which the term is used clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein. Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two or more 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).

2. TGF-beta Superfamily Type I Receptor and Type II Receptor Polypeptides and Heteromultimers Thereof

In certain aspects, the present disclosure relates to heteromultimers comprising one or more TGF-beta superfamily type I receptor polypeptides (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7 proteins from humans or other species such as those described herein, e.g., SEQ ID NOs: 14, 15, 124, 126, 413, 414, 18, 19, 136, 138, 421, 422, 22, 23, 115, 117, 407, 408, 26, 27, 83, 84, 104, 106, 403, 404, 30, 31, 87, 88, 139, 141, 423, 424, 34, 35, 91, 92, 142, 144, 425, 426, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 405, and 406) and one or more TGF-beta superfamily type II receptor polypeptides (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII proteins from humans or other species such as those described herein, e.g., SEQ ID NOs: 9, 10, 11, 118, 120, 409, 410, 1, 2, 3, 4, 5, 6, 100, 102, 401, 402, 46, 47, 71, 72, 121, 123, 411, 412, 50, 51, 75, 76, 79, 80, 42, 43, 67, 68, 127, 129, 130, 132, 415, 416, 417, and 418); heteromultimers comprising at least two different TGF-beta superfamily type I receptor polypeptides (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7 proteins from humans or other species such as those described herein, e.g., SEQ ID NOs: 14, 15, 124, 126, 413, 414, 18, 19, 136, 138, 421, 422, 22, 23, 115, 117, 407, 408, 26, 27, 83, 84, 104, 106, 403, 404, 30, 31, 87, 88, 139, 141, 423, 424, 34, 35, 91, 92, 142, 144, 425, 426, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 405, and 406); and heteromultimer complexes comprising at least two different TGF-beta superfamily type II receptor polypeptides (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII proteins from humans or other species such as those described herein, e.g., SEQ ID NOs: 9, 10, 11, 118, 120, 409, 410, 1, 2, 3, 4, 5, 6, 100, 102, 401, 402, 46, 47, 71, 72, 121, 123, 411, 412, 50, 51, 75, 76, 79, 80, 42, 43, 67, 68, 127, 129, 130, 132, , 415, 416, 417, and 418), which are generally referred to herein as "heteromers", "heteromultimer complexes" or "heteromultimers". Preferably, heteromultimers are soluble, *e.g.*, a heteromultimer comprises a soluble portion (domain) of at least one TGFβ superfamily type I receptor polypeptide and a soluble portion of at least one TGFβ superfamily type II receptor polypeptide. In general, the extracellular domains of TGFβ superfamily type I and type II receptors correspond to a soluble portion of the type I and type II receptor. Therefore, in some embodiments, heteromultimers of the disclosure comprise an extracellular domain of a TGFβ superfamily type I receptor polypeptide (*e.g.*, one or more ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and/or ALK7 receptor extracellular domains) and/or an extracellular domain of a TGFβ superfamily type II receptor polypeptide (*e.g.*, one or more ActRIIA, ActRIIB, TGFBRII, BMPRII, and/or MISRII receptor extracellular domains). Exemplary extracellular domains of ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII are disclosed herein and such sequences, as well

as fragments, functional variants, and modified forms thereof, may be used in accordance with the inventions of the present disclosure (*e.g.*, heteromultimers compositions and uses thereof). Heteromultimers of the disclosure include, e.g., heterodimers, heterotrimers, heterotetramers, and higher order oligomeric structures. See, e.g., Figures 1, 2, and 15. In certain preferred embodiments, heteromultimers of the disclosure are heterodimers.

A defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by 10, 12, or 14 conserved cysteine residues located at varying positions within the extracellular domain of each monomeric receptor. See, *e.g.*, Greenwald et al. (1999) Nat Struct Biol 6:18-22; Hinck (2012) FEBS Lett 586:1860-1870. The core ligand-binding domains of TGFβ superfamily receptors, as demarcated by the outermost of these conserved cysteines, corresponds to positions 29-109 of SEQ ID NO: 1 (ActRIIB precursor); positions 30-110 of SEQ ID NO: 9 (ActRIIA precursor); positions 34-95 of SEQ ID NO: 14 (ALK1 precursor); positions 35-99 of SEQ ID NO: 18 (ALK2 precursor); positions 61-130 of SEQ ID NO: 22 (ALK3 precursor); positions 34-101 of SEQ ID NOs: 26 and 83 (ALK4 precursors); positions 36-106 of SEQ ID NOs: 30 and 87 (ALK5 precursors); positions 32-102 of SEQ ID NO: 34 (ALK6 isoform B precursor); positions 28-92 of SEQ ID NOs: 38, 305, and 309 (ALK7 precursors); positions 51-143 of SEQ ID NO: 42 (TGFBRII isoform B precursor); positions 34-123 of SEQ ID NO: 46 and 71 (BMPRII precursors); positions 24-116 of SEQ ID NO: 50, 75, and 79 (MISRII precursors); positions 44-168 of SEQ ID NO: 67 (TGFBRII isoform A precursor); and positions 62-132 of SEQ ID NO: 91 (ALK6 isoform A precursor). The structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated on either terminus without necessarily altering ligand binding. Exemplary extracellular domains for N-terminal and/or C-terminal truncation include SEQ ID NOs: 2, 3, 5, 6, 10, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 51, 68, 72, 76, 80, 84, 88, 92, 302, 306, 310, and 313.

In preferred embodiments, heteromultimers of the disclosure bind to and/or inhibit (antagonize) activity of one or more TGF-beta superfamily ligands including, but not limited to, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty. In particular, heteromultimers of the disclosure may be used to antagonize signaling transduction (*e.g.*, Smad 2/3 and/or

Smad 1/5/8 signaling) initiated by one or more TGFβ superfamily ligands, which may be determined, for example, using a cell-based assay such as those described herein. As described herein, such antagonist heteromultimers may be useful in the treatment or prevention of various disorders/conditions associated with, *e.g.*, muscle loss, insufficient muscle growth, neurodegeneration, bone loss, reduced bone density and/or mineralization, insufficient bone growth, and/or obesity. In some embodiments, heteromultimers of the disclosure have different ligand binding specificities/profiles in comparison to their corresponding homomultimer complex (*e.g.*, an ALK4:ActRIIB heterodimer vs. a corresponding ActRIIB or ALK4 homodimer).

As used herein, the term "ActRIIB" refers to a family of activin receptor type IIB (ActRIIB) proteins from any species and variants derived from such ActRIIB proteins by mutagenesis or other modification. Reference to ActRIIB herein is understood to be a reference to any one of the currently identified forms. Members of the ActRIIB family are generally transmembrane proteins, composed of a ligand-binding extracellular domain comprising a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ActRIIB polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ActRIIB family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Examples of such variant ActRIIB polypeptides are provided throughout the present disclosure as well as in International Patent Application Publication Nos. WO 2006/012627, WO 2008/097541, and Wo 2010/151426, which are incorporated herein by reference in their entirety.

A human ActRIIB precursor protein sequence is as follows:

```
  1 MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
 51 GEQDKRLHCY ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
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451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501 TNVDLPPKES SI (SEQ ID NO: 1)

The signal peptide is indicated with a single underline; an extracellular domain is indicated in **bold** font; and the potential, endogenous N-linked glycosylation sites are indicated with a double underline.

A processed extracellular ActRIIB polypeptide sequence is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDD FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 2).

In some embodiments, the protein may be produced with an "SGR..." sequence at the N-terminus. The C-terminal "tail" of the extracellular domain is indicated by a single underline. The sequence with the "tail" deleted (a Δ15 sequence) is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDD FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO: 3).

A form of ActRIIB with an alanine at position 64 of SEQ ID NO: 1 (A64) is also reported in the literature. See, *e.g.*, Hilden *et al.* (1994) Blood, 83(8): 2163-2170. Applicants have ascertained that an ActRIIB-Fc fusion protein comprising an extracellular domain of ActRIIB with the A64 substitution has a relatively low affinity for activin and GDF11. By contrast, the same ActRIIB-Fc fusion protein with an arginine at position 64 (R64) has an affinity for activin and GDF11 in the low nanomolar to high picomolar range. Therefore, sequences with an R64 are used as the "wild-type" reference sequence for human ActRIIB in this disclosure.

A form of ActRIIB with an alanine at position 64 is as follows:

1 MTAPWVALAL LWGSLCAGS**G RGEAETRECI YYNANWELER TNQSGLERCE**
51 **GEQDKRLHCY ASWANSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY**
101 **FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPT**LLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL

451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501 TNVDLPPKES SI (SEQ ID NO: 4)

The signal peptide is indicated by single underline and the extracellular domain is indicated by **bold** font.

A processed extracellular ActRIIB polypeptide sequence of the alternative A64 form is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDD
FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID
NO: 5)

In some embodiments, the protein may be produced with an "SGR..." sequence at the N-terminus. The C-terminal "tail" of the extracellular domain is indicated by single underline. The sequence with the "tail" deleted (a Δ15 sequence) is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDD
FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO: 6)

A nucleic acid sequence encoding the human ActRIIB precursor protein is shown in SEQ ID NO: 7, representing nucleotides 25-1560 of Genbank Reference Sequence NM_001106.3, which encode amino acids 1-513 of the ActRIIB precursor. The sequence as shown in SEQ ID NO: 7 provides an arginine at position 64 and may be modified to provide an alanine instead. A nucleic acid sequence encoding a processed extracellular human ActRIIB polypeptide is shown in SEQ ID NO: 8. The sequence of SEQ ID NO: 8 provides an arginine at position 64, and may be modified to provide an alanine instead.

An alignment of the amino acid sequences of human ActRIIB extracellular domain and human ActRIIA extracellular domain are illustrated in Figure 3. This alignment indicates amino acid residues within both receptors that are believed to directly contact ActRII ligands. For example, the composite ActRII structures indicated that the ActRIIB-ligand binding pocket is defined, in part, by residues Y31, N33, N35, L38 through T41, E47, E50, Q53 through K55, L57, H58, Y60, S62, K74, W78 through N83, Y85, R87, A92, and E94 through F101. At these positions, it is expected that conservative mutations will be tolerated.

In addition, ActRIIB is well-conserved among vertebrates, with large stretches of the extracellular domain completely conserved. For example, Figure 4 depicts a multi-sequence alignment of a human ActRIIB extracellular domain compared to various ActRIIB orthologs. Many of the ligands that bind to ActRIIB are also highly conserved. Accordingly, from these

alignments, it is possible to predict key amino acid positions within the ligand-binding domain that are important for normal ActRIIB-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant of substitution without significantly altering normal ActRIIB-ligand binding activities. Therefore, an active, human ActRIIB variant polypeptide useful in accordance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ActRIIB, or may include a residue that is similar to that in the human or other vertebrate sequences. Without meaning to be limiting, the following examples illustrate this approach to defining an active ActRIIB variant. L46 in the human extracellular domain (SEQ ID NO: 2) is a valine in *Xenopus* ActRIIB (SEQ ID NO: 506), and so this position may be altered, and optionally may be altered to another hydrophobic residue, such as V, I or F, or a non-polar residue such as A. E52 in the human extracellular domain is a K in *Xenopus*, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y and probably A. T93 in the human extracellular domain is a K in *Xenopus*, indicating that a wide structural variation is tolerated at this position, with polar residues favored, such as S, K, R, E, D, H, G, P, G and Y. F108 in the human extracellular domain is a Y in *Xenopus*, and therefore Y or other hydrophobic group, such as I, V or L should be tolerated. E111 in the human extracellular domain is K in *Xenopus*, indicating that charged residues will be tolerated at this position, including D, R, K and H, as well as Q and N. R112 in the human extracellular domain is K in *Xenopus*, indicating that basic residues are tolerated at this position, including R and H. A at position 119 in the human extracellular domain is relatively poorly conserved, and appears as P in rodents and V in *Xenopus*, thus essentially any amino acid should be tolerated at this position.

Moreover, ActRII proteins have been characterized in the art in terms of structural and functional characteristics, particularly with respect to ligand binding [Attisano *et al.* (1992) Cell 68(1):97-108; Greenwald *et al.* (1999) Nature Structural Biology 6(1): 18-22; Allendorph *et al.* (2006) PNAS 103(20: 7643-7648; Thompson *et al.* (2003) The EMBO Journal 22(7): 1555-1566; as well as U.S. Patent Nos: 7,709,605, 7,612,041, and 7,842,663]. In addition to the teachings herein, these references provide amply guidance for how to generate ActRIIB variants that retain one or more normal activities (e.g., ligand-binding activity).

For example, a defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by conserved

cysteine residues located at varying positions within the extracellular domain of each monomeric receptor [Greenwald et al. (1999) Nat Struct Biol 6:18-22; and Hinck (2012) FEBS Lett 586:1860-1870]. Accordingly, the core ligand-binding domains of human ActRIIB, as demarcated by the outermost of these conserved cysteines, corresponds to positions 29-109 of SEQ ID NO: 1 (ActRIIB precursor). Thus, the structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 residues at the N-terminus and/or by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues a the C-terminus without necessarily altering ligand binding. Exemplary ActRIIB extracellular domains for N-terminal and/or C-terminal truncation include SEQ ID NOs: 2, 3, 5, and 6.

Attisano *et al.* showed that a deletion of the proline knot at the C-terminus of the extracellular domain of ActRIIB reduced the affinity of the receptor for activin. An ActRIIB-Fc fusion protein containing amino acids 20-119 of present SEQ ID NO: 1, "ActRIIB(20-119)-Fc", has reduced binding to GDF11 and activin relative to an ActRIIB(20-134)-Fc, which includes the proline knot region and the complete juxtamembrane domain (see, *e.g.*, U.S. Patent No. 7,842,663). However, an ActRIIB(20-129)-Fc protein retains similar, but somewhat reduced activity, relative to the wild-type, even though the proline knot region is disrupted.

Thus, ActRIIB extracellular domains that stop at amino acid 134, 133, 132, 131, 130 and 129 (with respect to SEQ ID NO: 1) are all expected to be active, but constructs stopping at 134 or 133 may be most active. Similarly, mutations at any of residues 129-134 (with respect to SEQ ID NO: 1) are not expected to alter ligand-binding affinity by large margins. In support of this, it is known in the art that mutations of P129 and P130 (with respect to SEQ ID NO: 1) do not substantially decrease ligand binding. Therefore, an ActRIIB polypeptide of the present disclosure may end as early as amino acid 109 (the final cysteine), however, forms ending at or between 109 and 119 (*e.g.*, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119) are expected to have reduced ligand binding. Amino acid 119 (with respect to present SEQ ID NO: 1) is poorly conserved and so is readily altered or truncated. ActRIIB polypeptides ending at 128 (with respect to SEQ ID NO: 1) or later should retain ligand-binding activity. ActRIIB polypeptides ending at or between 119 and 127 (*e.g.*, 119, 120, 121, 122, 123, 124, 125, 126, or 127), with respect to SEQ ID NO: 1, will have an

intermediate binding ability. Any of these forms may be desirable to use, depending on the clinical or experimental setting.

At the N-terminus of ActRIIB, it is expected that a protein beginning at amino acid 29 or before (with respect to SEQ ID NO: 1) will retain ligand-binding activity. Amino acid 29 represents the initial cysteine. An alanine-to-asparagine mutation at position 24 (with respect to SEQ ID NO: 1) introduces an N-linked glycosylation sequence without substantially affecting ligand binding [U.S. Patent No. 7,842,663]. This confirms that mutations in the region between the signal cleavage peptide and the cysteine cross-linked region, corresponding to amino acids 20-29, are well tolerated. In particular, ActRIIB polypeptides beginning at position 20, 21, 22, 23, and 24 (with respect to SEQ ID NO: 1) should retain general ligand-biding activity, and ActRIIB polypeptides beginning at positions 25, 26, 27, 28, and 29 (with respect to SEQ ID NO: 1) are also expected to retain ligand-biding activity. It has been demonstrated, *e.g.*, U.S. Patent No. 7,842,663, that, surprisingly, an ActRIIB construct beginning at 22, 23, 24, or 25 will have the most activity.

Taken together, a general formula for an active portion (*e.g.*, ligand-binding portion) of ActRIIB comprises amino acids 29-109 of SEQ ID NO: 1. Therefore ActRIIB polypeptides may, for example, comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to any one of amino acids 20-29 (*e.g.*, beginning at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and ending at a position corresponding to any one amino acids 109-134 (*e.g.*, ending at any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Other examples include polypeptides that begin at a position from 20-29 (*e.g.*, any one of positions 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) or 21-29 (*e.g.*, any one of positions 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and end at a position from 119-134 (*e.g.*, any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-133 (*e.g.*, any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133), 129-134 (*e.g.*, any one of positions 129, 130, 131, 132, 133, or 134), or 129-133 (*e.g.*, any one of positions 129, 130, 131, 132, or 133) of SEQ ID NO: 1. Other examples include constructs that begin at a position from 20-24 (*e.g.*, any one of positions 20, 21, 22, 23, or 24), 21-24 (*e.g.*, any one of positions 21, 22, 23, or 24), or 22-25 (*e.g.*, any one of positions 22, 22, 23, or 25) of SEQ ID NO: 1 and end at a position

from 109-134 (*e.g.*, any one of positions 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-134 (*e.g.*, any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) or 129-134 (*e.g.*, any one of positions 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Variants within these ranges are also contemplated, particularly those having at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 1.

The variations described herein may be combined in various ways. In some embodiments, ActRIIB variants comprise no more than 1, 2, 5, 6, 7, 8, 9, 10 or 15 conservative amino acid changes in the ligand-binding pocket, and zero, one, or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand-binding pocket. Sites outside the binding pocket, at which variability may be particularly well tolerated, include the amino and carboxy termini of the extracellular domain (as noted above), and positions 42-46 and 65-73 (with respect to SEQ ID NO: 1). An asparagine-to-alanine alteration at position 65 (N65A) actually improves ligand binding in the A64 background, and is thus expected to have no detrimental effect on ligand binding in the R64 background [U.S. Patent No. 7,842,663]. This change probably eliminates glycosylation at N65 in the A64 background, thus demonstrating that a significant change in this region is likely to be tolerated. While an R64A change is poorly tolerated, R64K is well-tolerated, and thus another basic residue, such as H may be tolerated at position 64 [U.S. Patent No. 7,842,663]. Additionally, the results of the mutagenesis program described in the art indicate that there are amino acid positions in ActRIIB that are often beneficial to conserve. With respect to SEQ ID NO: 1, these include position 80 (acidic or hydrophobic amino acid), position 78 (hydrophobic, and particularly tryptophan), position 37 (acidic, and particularly aspartic or glutamic acid), position 56 (basic amino acid), position 60 (hydrophobic amino acid, particularly phenylalanine or tyrosine). Thus, the disclosure provides a framework of amino acids that may be conserved in ActRIIB polypeptides. Other positions that may be desirable to conserve are as follows: position 52 (acidic amino acid), position 55 (basic amino acid), position 81 (acidic), 98 (polar or charged, particularly E, D, R or K), all with respect to SEQ ID NO: 1.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ActRIIB polypeptides for use in accordance with the disclosure

are soluble (*e.g.*, an extracellular domain of ActRIIB). In other preferred embodiments, ActRIIB polypeptides for use in accordance with the disclosure bind to one or more TGF-beta superfamily ligands. Therefore, in some embodiments, ActRIIB polypeptides for use in accordance with the disclosure inhibit (antagonize) activity (*e.g.*, inhibition of Smad signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to amino acids 20-29 (*e.g.*, beginning at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and ending at a position corresponding to amino acids 109-134 (*e.g.*, ending at any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. In certain preferred embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 29-109 of SEQ ID NO: 1 In other preferred embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 25-131 of SEQ ID NO: 1 In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 401, and 402. In certain embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide wherein the amino acid position corresponding to L79 of SEQ ID NO: 1 is not an acidic amino acid (*i.e.*, is not a naturally occurring D or E amino acid residue or artificial acidic amino acid).

In certain embodiments, the present disclosure relates to a protein complex comprising an ActRIIA polypeptide. As used herein, the term "ActRIIA" refers to a family of activin receptor type IIA (ActRIIA) proteins from any species and variants derived from such ActRIIA proteins by mutagenesis or other modification. Reference to ActRIIA herein is understood to be a reference to any one of the currently identified forms. Members of the ActRIIA family are generally transmembrane proteins, composed of a ligand-binding

extracellular domain comprising a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ActRIIA polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ActRIIA family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Examples of such variant ActRIIA polypeptides are provided throughout the present disclosure as well as in International Patent Application Publication No. WO 2006/012627, which is incorporated herein by reference in its entirety.

The human ActRIIA precursor protein sequence is as follows:

```
  1 MGAAAKLAFA VFLISCSSGA ILGRSETQEC LFFNANWEKD RTNQTGVEPC
 51 YGDKDKRRHC FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKKDSPEV
101 YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPPYYNIL LYSLVPLMLI
151 AGIVICAFWV YRHHKMAYPP VLVPTQDPGP PPPSPLLGLK PLQLLEVKAR
201 GRFGCVWKAQ LLNEYVAVKI FPIQDKQSWQ NEYEVYSLPG MKHENILQFI
251 GAEKRGTSVD VDLWLITAFH EKGSLSDFLK ANVVSWNELC HIAETMARGL
301 AYLHEDIPGL KDGHKPAISH RDIKSKNVLL KNNLTACIAD FGLALKFEAG
351 KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR
401 CTAADGPVDE YMLPFEEEIG QHPSLEDMQE VVVHKKKRPV LRDYWQKHAG
451 MAMLCETIEE CWDHDAEARL SAGCVGERIT QMQRLTNIIT TEDIVTVVTM
501 VTNVDFPPKE SSL  (SEQ ID NO: 9)
```

The signal peptide is indicated by a single underline; the extracellular domain is indicated in **bold** font; and the potential, endogenous N-linked glycosylation sites are indicated by a double underline.

The processed extracellular human ActRIIA polypeptide sequence is as follows:

```
ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDD
INCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPP  (SEQ ID
NO: 10)
```

The C-terminal "tail" of the extracellular domain is indicated by a single underline. The sequence with the "tail" deleted (a Δ15 sequence) is as follows:

```
ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDD
INCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEM (SEQ ID NO: 11)
```

A nucleic acid sequence encoding the human ActRIIA precursor protein is shown in SEQ ID NO: 12, corresponding to nucleotides 159-1700 of Genbank Reference Sequence NM_001616.4. A nucleic acid sequence encoding a processed extracellular ActRIIA polypeptide is as shown in SEQ ID NO: 13.

A general formula for an active (*e.g.*, ligand binding) ActRIIA polypeptide is one that comprises a polypeptide that starts at amino acid 30 and ends at amino acid 110 of SEQ ID NO: 9. Accordingly, ActRIIA polypeptides of the present disclosure may comprise a polypeptide that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9. Optionally, ActRIIA polypeptides of the present disclosure comprise a polypeptide that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids amino acids 12-82 of SEQ ID NO: 9 optionally beginning at a position ranging from 1-5 (*e.g.*, 1, 2, 3, 4, or 5) or 3-5 (*e.g.*, 3, 4, or 5) and ending at a position ranging from 110-116 (*e.g.*, 110, 111, 112, 113, 114, 115, or 116) or 110-115 (*e.g.*, 110, 111, 112, 113, 114, or 115), respectively, and comprising no more than 1, 2, 5, 10 or 15 conservative amino acid changes in the ligand binding pocket, and zero, one or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand-binding pocket with respect to SEQ ID NO: 9.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ActRIIA polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ActRIIA polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ActRIIA). In other preferred embodiments, ActRIIA polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIA polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 409, or 410. In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIA polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 409, or 410.

In certain aspects, the present disclosure relates to protein complexes that comprise a TGFBRII polypeptide. As used herein, the term "TGFBRII" refers to a family of

transforming growth factor-beta receptor II (TGFBRII) proteins from any species and variants derived from such proteins by mutagenesis or other modification. Reference to TGFBRII herein is understood to be a reference to any one of the currently identified forms. Members of the TGFBRII family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "TGFBRII polypeptide" includes polypeptides comprising any naturally occurring polypeptide of a TGFBRII family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

A human TGFBRII precursor protein sequence (NCBI Ref Seq NP_003233.4) is as follows:

```
  1 MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL
 51 CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
101 CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS
151 EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI SVIIIFYCYR VNRQQKLSST
201 WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE LLPIELDTLV
251 GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
301 HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL
351 GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
401 SLRLDPTLSV DDLANSGQVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM
451 ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI
501 PSFWLNHQGI QMVCETLTEC WDHDPEARLT AQCVAERFSE LEHLDRLSGR
551 SCSEEKIPED GSLNTTK     (SEQ ID NO: 42)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular TGFBRII polypeptide sequence is as follows:

```
TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVC
VAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDN
IIFSEEYNTSNPDLLLVIFQ (SEQ ID NO: 43)
```

A nucleic acid sequence encoding TGFBRII precursor protein is shown in SEQ ID NO:44, corresponding to nucleotides 383-2083 of Genbank Reference Sequence

NM_003242.5. A nucleic acid sequence encoding a processed extracellular TGFBRII polypeptide is shown in SEQ ID NO: 45.

An alternative isoform of TGFBRII, isoform A (NP_001020018.1), is as follows:

```
  1 MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT
 51 AHPLRHINND MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS
101 ICEKPQEVCV AVWRKNDENI TLETVCHDPK LPYHDFILED AASPKCIMKE
151 KKKPGETFFM CSCSSDECND NIIFSEEYNT SNPDLLLVIF QVTGISLLPP
201 LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH CAIILEDDRS
251 DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA
301 VKIFPYEEYA SWKTEKDIFS DINLKHENIL QFLTAEERKT ELGKQYWLIT
351 AFHAKGNLQE YLTRHVISWE DLRKLGSSLA RGIAHLHSDH TPCGRPKMPI
401 VHRDLKSSNI LVKNDLTCCL CDFGLSLRLD PTLSVDDLAN SGQVGTARYM
451 APEVLESRMN LENVESFKQT DVYSMALVLW EMTSRCNAVG EVKDYEPPFG
501 SKVREHPCVE SMKDNVLRDR GRPEIPSFWL NHQGIQMVCE TLTECWDHDP
551 EARLTAQCVA ERFSELEHLD RLSGRSCSEE KIPEDGSLNT TK
    (SEQ ID NO: 67)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular TGFBRII polypeptide sequence (isoform A) is as follows:

```
TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFS
TCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKC
IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQ (SEQ ID NO: 68)
```

A nucleic acid sequence encoding the TGFBRII precursor protein (isoform A) is shown in SEQ ID NO: 69, corresponding to nucleotides 383-2158 of Genbank Reference Sequence NM_001024847.2. A nucleic acid sequence encoding the processed extracellular TGFBRII polypeptide (isoform A) is shown in SEQ ID NO: 70.

Either of the foregoing TGFβRII isoforms (SEQ ID NOs: 42, 43, 67, and 68) could incorporate an insertion of 36 amino acids (SEQ ID NO: 95) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 42; positions 129 and 130 of SEQ ID NO: 43; positions 176 and 177 of SEQ ID NO: 67; or positions 154 and 155 of SEQ ID NO: 68)

located near the C-terminus of the TGFβRII ECD, as occurs naturally in the TGFβRII isoform C (Konrad et al., BMC Genomics 8:318, 2007).

GRCKIRHIGS NNRLQRSTCQ NTGWESAHVM KTPGFR (SEQ ID NO: 95)

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, TGFBRII polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising a TGFBRII polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of TGFBRII). In other preferred embodiments, TGFBRII polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one TGFBRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 42, 43, 67, or 68, with or without insertion of SEQ ID NO: 95 as described above. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one TGFBRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 42, 43, 67, or 68, with or without insertion of SEQ ID NO: 95.

In certain aspects, the present disclosure relates to protein complexes that comprise a BMPRII polypeptide. As used herein, the term "BMPRII" refers to a family of bone morphogenetic protein receptor type II (BMPRII) proteins from any species and variants derived from such BMPRII proteins by mutagenesis or other modification. Reference to BMPRII herein is understood to be a reference to any one of the currently identified forms. Members of the BMPRII family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "BMPRII polypeptide" includes polypeptides comprising any naturally occurring polypeptide of a BMPRII family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

A human BMPRII precursor protein sequence (NCBI Ref Seq NP_001195.2) is as follows:

1 MTSSLQRPWR VPWLPWTILL VSTAAASQNQ ERLCAFKDPY QQDLGIGESR

```
  51 ISHENGTILC SKGSTCYGLW EKSKGDINLV KQGCWSHIGD PQECHYEECV
 101 VTTTPPSIQN GTYRFCCCST DLCNVNFTEN FPPPDTTPLS PPHSFNRDET
 151 IIIALASVSV LAVLIVALCF GYRMLTGDRK QGLHSMNMME AAASEPSLDL
 201 DNLKLLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNF INEKNIYRVP
 251 LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS
 301 SCRLAHSVTR GLAYLHTELP RGDHYKPAIS HRDLNSRNVL VKNDGTCVIS
 351 DFGLSMRLTG NRLVRPGEED NAAISEVGTI RYMAPEVLEG AVNLRDCESA
 401 LKQVDMYALG LIYWEIFMRC TDLFPGESVP EYQMAFQTEV GNHPTFEDMQ
 451 VLVSREKQRP KFPEAWKENS LAVRSLKETI EDCWDQDAEA RLTAQCAEER
 501 MAELMMIWER NKSVSPTVNP MSTAMQNERN LSHNRRVPKI GPYPDYSSSS
 551 YIEDSIHHTD SIVKNISSEH SMSSTPLTIG EKNRNSINYE RQQAQARIPS
 601 PETSVTSLST NTTTTNTTGL TPSTGMTTIS EMPYPDETNL HTTNVAQSIG
 651 PTPVCLQLTE EDLETNKLDP KEVDKNLKES SDENLMEHSL KQFSGPDPLS
 701 STSSSLLYPL IKLAVEATGQ QDFTQTANGQ ACLIPDVLPT QIYPLPKQQN
 751 LPKRPTSLPL NTKNSTKEPR LKFGSKHKSN LKQVETGVAK MNTINAAEPH
 801 VVTVTMNGVA GRNHSVNSHA ATTQYANGTV LSGQTTNIVT HRAQEMLQNQ
 851 FIGEDTRLNI NSSPDEHEPL LRREQQAGHD EGVLDRLVDR RERPLEGGRT
 901 NSNNNNSNPC SEQDVLAQGV PSTAADPGPS KPRRAQRPNS LDLSATNVLD
 951 GSSIQIGEST QDGKSGSGEK IKKRVKTPYS LKRWRPSTWV ISTESLDCEV
1001 NNNGSNRAVH SKSSTAVYLA EGGTATTMVS KDIGMNCL
```

(SEQ ID NO: 46)

The signal peptide is indicated by a single underline and an extracellular domain is indicated in **bold** font.

A processed extracellular BMPRII polypeptide sequence is as follows:

SQNQERLCAFKDPYQQDLGIGESRISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHI
GDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET
(SEQ ID NO: 47)

A nucleic acid sequence encoding BMPRII precursor protein is shown in SEQ ID NO: 48, as follows nucleotides 1149-4262 of Genbank Reference Sequence NM_001204.6. A nucleic acid sequence encoding an extracellular BMPRII polypeptide is shown in SEQ ID NO: 49.

An alternative isoform of BMPRII, isoform 2 (GenBank: AAA86519.1) is as follows:

MTSSLQRPWR VPWLPWTILL VSTAAA**SQNQ ERLCAFKDPY QQDLGIGESR**
ISHENGTILC SKGSTCYGLW EKSKGDINLV KQGCWSHIGD PQECHYEECV
VTTTPPSIQN GTYRFCCCST DLCNVNFTEN FPPPDTTPLS PPHSFNRDET
IIIALASVSV LAVLIVALCF GYRMLTGDRK QGLHSMNMME AAASEPSLDL
DNLKLLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNF INEKNIYRVP
LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS
SCRLAHSVTR GLAYLHTELP RGDHYKPAIS HRDLNSRNVL VKNDGTCVIS
DFGLSMRLTG NRLVRPGEED NAAISEVGTI RYMAPEVLEG AVNLRDCESA
LKQVDMYALG LIYWEIFMRC TDLFPGESVP EYQMAFQTEV GNHPTFEDMQ
VLVSREKQRP KFPEAWKENS LAVRSLKETI EDCWDQDAEA RLTAQCAEER
MAELMMIWER NKSVSPTVNP MSTAMQNERR (SEQ ID NO: 71)

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular BMPRII polypeptide sequence (isoform 2) is as follows:

SQNQERLCAFKDPYQQDLGIGESRISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHI
GDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET
(SEQ ID NO: 72)

A nucleic acid sequence encoding human BMPRII precursor protein (isoform 2) is shown in SEQ ID NO:73, corresponding to nucleotides 163-1752 of Genbank Reference Sequence U25110.1. The signal sequence is underlined. A nucleic acid sequence encoding an extracellular BMPRII polypeptide (isoform 2) is shown in SEQ ID NO: 74

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, BMPRII polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising a BMPRII polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of BMPRII). In other preferred embodiments, BMPRII polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the

disclosure comprise at least one BMPRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 46, 47, 71, 72, 121, 123, 411, or 412. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one BMPRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 46, 47, 71, 72, 121, 123, 411, or 412.

In certain aspects, the present disclosure relates to protein complexes that comprise an MISRII polypeptide. As used herein, the term "MISRII" refers to a family of Müllerian inhibiting substance receptor type II (MISRII) proteins from any species and variants derived from such MISRII proteins by mutagenesis or other modification. Reference to MISRII herein is understood to be a reference to any one of the currently identified forms. Members of the MISRII family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "MISRII polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an MISRII family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

A human MISRII precursor protein sequence (NCBI Ref Seq NP_065434.1) is as follows:

```
  1 MLGSLGLWAL LPTAVEAPPN RRTCVFFEAP GVRGSTKTLG ELLDTGTELP
 51 RAIRCLYSRC CFGIWNLTQD RAQVEMQGCR DSDEPGCESL HCDPSPRAHP
101 SPGSTLFTCS CGTDFCNANY SHLPPPGSPG TPGSQGPQAA PGESIWMALV
151 LLGLFLLLLL LLGSIILALL QRKNYRVRGE PVPEPRPDSG RDWSVELQEL
201 PELCFSQVIR EGGHAVVWAG QLQGKLVAIK AFPPRSVAQF QAERALYELP
251 GLQHDHIVRF ITASRGGPGR LLSGPLLVLE LHPKGSLCHY LTQYTSDWGS
301 SLRMALSLAQ GLAFLHEERW QNGQYKPGIA HRDLSSQNVL IREDGSCAIG
351 DLGLALVLPG LTQPPAWTPT QPQGPAAIME AGTQRYMAPE LLDKTLDLQD
401 WGMALRRADI YSLALLLWEI LSRCPDLRPD SSPPPFQLAY EAELGNTPTS
451 DELWALAVQE RRRPYIPSTW RCFATDPDGL RELLEDCWDA DPEARLTAEC
501 VQQRLAALAH PQESHPFPES CPRGCPPLCP EDCTSIPAPT ILPCRPQRSA
551 CHFSVQQGPC SRNPQPACTL SPV (SEQ ID NO: 50)
```

The signal peptide is indicated by a single underline and an extracellular domain is indicated in **bold** font.

A processed extracellular MISRII polypeptide sequence is as follows:

PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
RDSDEPGCESLHCDPSPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
GESIWMAL (SEQ ID NO: 51)

A nucleic acid sequence encoding the MISRII precursor protein is shown in SEQ ID NO: 52, corresponding to nucleotides 81-1799 of Genbank Reference Sequence NM_020547.2. A nucleic acid sequence encoding the extracellular human MISRII polypeptide is shown in SEQ ID NO: 53.

An alternative isoform of the human MISRII precursor protein sequence, isoform 2 (NCBI Ref Seq NP_001158162.1), is as follows:

1 MLGSLGLWAL LPTAVEA**PPN RRTCVFFEAP GVRGSTKTLG ELLDTGTELP**
051 **RAIRCLYSRC CFGIWNLTQD RAQVEMQGCR DSDEPGCESL HCDPSPRAHP**
101 **SPGSTLFTCS CGTDFCNANY SHLPPPGSPG TPGSQGPQAA PGESIWMAL**V
151 LLGLFLLLLL LLGSIILALL QRKNYRVRGE PVPEPRPDSG RDWSVELQEL
201 PELCFSQVIR EGGHAVVWAG QLQGKLVAIK AFPPRSVAQF QAERALYELP
251 GLQHDHIVRF ITASRGGPGR LLSGPLLVLE LHPKGSLCHY LTQYTSDWGS
301 SLRMALSLAQ GLAFLHEERW QNGQYKPGIA HRDLSSQNVL IREDGSCAIG
351 DLGLALVLPG LTQPPAWTPT QPQGPAAIME AGTQRYMAPE LLDKTLDLQD
401 WGMALRRADI YSLALLLWEI LSRCPDLRPA VHHPSNWPMR QNWAIPLPLM
451 SYGPWQCRRG GVPTSHPPGA ALPQTLMG (SEQ ID NO: 75)

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular MISRII polypeptide sequence (isoform 2) is as follows:
PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
RDSDEPGCESLHCDPSPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
GESIWMAL (SEQ ID NO: 76)

A nucleic acid sequence encoding the MISRII precursor protein (isoform 2) is shown in SEQ ID NO: 77, corresponding to nucleotides 81-1514 of Genbank Reference Sequence NM_001164690.1. A nucleic acid sequence encoding processed soluble (extracellular) human MISRII polypeptide (isoform 2) is shown in SEQ ID NO: 78.

An alternative isoform of the human MISRII precursor protein sequence, isoform 3 (NCBI Ref Seq NP_001158163.1), is as follows:

1 MLGSLGLWAL LPTAVEAPPN RRTCVFFEAP GVRGSTKTLG ELLDTGTELP
51 RAIRCLYSRC CFGIWNLTQD RAQVEMQGCR DSDEPGCESL HCDPSPRAHP
101 SPGSTLFTCS CGTDFCNANY SHLPPPGSPG TPGSQGPQAA PGESIWMALV
151 LLGLFLLLLL LLGSIILALL QRKNYRVRGE PVPEPRPDSG RDWSVELQEL
201 PELCFSQVIR EGGHAVVWAG QLQGKLVAIK AFPPRSVAQF QAERALYELP
251 GLQHDHIVRF ITASRGGPGR LLSGPLLVLE LHPKGSLCHY LTQYTSDWGS
301 SLRMALSLAQ GLAFLHEERW QNGQYKPGIA HRDLSSQNVL IREDGSCAIG
351 DLGLALVLPG LTQPPAWTPT QPQGPAAIME DPDGLRELLE DCWDADPEAR
401 LTAECVQQRL AALAHPQESH PFPESCPRGC PPLCPEDCTS IPAPTILPCR
451 PQRSACHFSV QQGPCSRNPQ PACTLSPV(SEQ ID NO: 79)

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular MISRII polypeptide sequence (isoform 3) is as follows:
PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
RDSDEPGCESLHCDPSPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
GESIWMAL (SEQ ID NO: 80)

A nucleic acid sequence encoding human MISRII precursor protein (isoform 3) is shown in SEQ ID NO: 81, corresponding to nucleotides 81-1514 of Genbank Reference Sequence NM_001164691.1. A nucleic acid sequence encoding a processed soluble (extracellular) human MISRII polypeptide (isoform 3) is shown in SEQ ID NO: 82.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, MISRII polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising a MISRII polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of MISRII). In other preferred embodiments, MISRII polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one MISRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 50, 51, 75, 76, 79, or 80. In some embodiments, heteromultimers of the disclosure consist or

consist essentially of at least one MISRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 50, 51, 75, 76, 79, or 80.

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK1 polypeptide. As used herein, the term "ALK1" refers to a family of activin receptor-like kinase-1 proteins from any species and variants derived from such ALK1 proteins by mutagenesis or other modification. Reference to ALK1 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK1 family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ALK1 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK1 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

The human ALK1 precursor protein sequence (NCBI Ref Seq NP_000011.2) is as follows:

1 MTLGSPRKGL LMLLMALVTQ GDPVKPSRGP LVTCTCESPH CKGPTCRGAW
51 CTVVLVREEG RHPQEHRGCG NLHRELCRGR PTEFVNHYCC DSHLCNHNVS
101 LVLEATQPPS EQPGTDGQLA LILGPVLALL ALVALGVLGL WHVRRRQEKQ
151 RGLHSELGES SLILKASEQG DSMLGDLLDS DCTTGSGSGL PFLVQRTVAR
201 QVALVECVGK GRYGEVWRGL WHGESVAVKI FSSRDEQSWF RETEIYNTVL
251 LRHDNILGFI ASDMTSRNSS TQLWLITHYH EHGSLYDFLQ RQTLEPHLAL
301 RLAVSAACGL AHLHVEIFGT QGKPAIAHRD FKSRNVLVKS NLQCCIADLG
351 LAVMHSQGSD YLDIGNNPRV GTKRYMAPEV LDEQIRTDCF ESYKWTDIWA
401 FGLVLWEIAR RTIVNGIVED YRPPFYDVVP NDPSFEDMKK VVCVDQQTPT
451 IPNRLAADPV LSGLAQMMRE CWYPNPSARL TALRIKKTLQ KISNSPEKPK
501 VIQ (SEQ ID NO: 14)

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK1 polypeptide sequence is as follows:

DPVKPSRGPLVTCTCESPHCKGPTCRGAWCTVVLVREEGRHPQEHRGCGNLHRELCRGRPTE
FVNHYCCDSHLCNHNVSLVLEATQPPSEQPGTDGQ (SEQ ID NO: 15)

A nucleic acid sequence encoding human ALK1 precursor protein is shown in SEQ ID NO: 16, corresponding to nucleotides 284-1792 of Genbank Reference Sequence NM_000020.2. A nucleic acid sequence encoding a processed extracelluar ALK1 polypeptide is in SEQ ID NO: 17.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK1 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK1 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK1). In other preferred embodiments, ALK1 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK1 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 14, 15, 124, 126, 413, or 414. In some embodiments, heteromultimers of the disclosure consist or consist essentially of at least one ALK1 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 14, 15, 124, 126, 413, or 414.

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK2 polypeptide. As used herein, the term "ALK2" refers to a family of activin receptor-like kinase-2 proteins from any species and variants derived from such ALK2 proteins by mutagenesis or other modification. Reference to ALK2 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK2 family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ALK2 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK2 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

A human ALK2 precursor protein sequence (NCBI Ref Seq NP_001096.1) is as follows:

1 MVDGVMILPV LIMIALPSPS MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG

51 **QQCFSSLSIN DGFHVYQKGC FQVYEQGKMT CKTPPSPGQA VECCQGDWCN**
101 **RNITAQLPTK GKSFPGTQNF HLE**VGLIILS VVFAVCLLAC LLGVALRKFK
151 RRNQERLNPR DVEYGTIEGL ITTNVGDSTL ADLLDHSCTS GSGSGLPFLV
201 QRTVARQITL LECVGKGRYG EVWRGSWQGE NVAVKIFSSR DEKSWFRETE
251 LYNTVMLRHE NILGFIASDM TSRHSSTQLW LITHYHEMGS LYDYLQLTTL
301 DTVSCLRIVL SIASGLAHLH IEIFGTQGKP AIAHRDLKSK NILVKKNGQC
351 CIADLGLAVM HSQSTNQLDV GNNPRVGTKR YMAPEVLDET IQVDCFDSYK
401 RVDIWAFGLV LWEVARRMVS NGIVEDYKPP FYDVVPNDPS FEDMRKVVCV
451 DQQRPNIPNR WFSDPTLTSL AKLMKECWYQ NPSARLTALR IKKTLTKIDN
501 SLDKLKTDC (SEQ ID NO: 18)

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK2 polypeptide sequence is as follows:

MEDEKPKVNPKLYMCVCEGLSCGNEDHCEGQQCFSSLSINDGFHVYQKGCFQVYEQGKMTCK
TPPSPGQAVECCQGDWCNRNITAQLPTKGKSFPGTQNFHLE (SEQ ID NO: 19)

A nucleic acid sequence encoding human ALK2 precursor protein is shown in SEQ ID NO: 20, corresponding to nucleotides 431-1957 of Genbank Reference Sequence NM_001105.4. A nucleic acid sequence encoding the extracellular ALK2 polypeptide is as in SEQ ID NO: 21.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK2 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK2 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK2). In other preferred embodiments, ALK2 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 18 or 19. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one ALK2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 18 or 19.

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK3 polypeptide. As used herein, the term "ALK3" refers to a family of activin receptor-like kinase-3 proteins from any species and variants derived from such ALK3 proteins by mutagenesis or other modification. Reference to ALK3 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK3 family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ALK3 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK3 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

A human ALK3 precursor protein sequence (NCBI Ref Seq NP_004320.2) is as follows:

```
  1  MPQLYIYIRL LGAYLFIISR VQGQNLDSML HGTGMKSDSD QKKSENGVTL APEDTLPFLK
 61  CYCSGHCPDD AINNTCITNG HCFAIIEEDD QGETTLASGC MKYEGSDFQC KDSPKAQLRR
121  TIECCRTNLC NQYLQPTLPP VVIGPFFDGS IRWLVLLISM AVCIIAMIIF SSCFCYKHYC
181  KSISSRRRYN RDLEQDEAFI PVGESLKDLI DQSQSSGSGS GLPLLVQRTI AKQIQMVRQV
241  GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT VLMRHENILG FIAADIKGTG
301  SWTQLYLITD YHENGSLYDF LKCATLDTRA LLKLAYSAAC GLCHLHTEIY GTQGKPAIAH
361  RDLKSKNILI KKNGSCCIAD LGLAVKFNSD TNEVDVPLNT RVGTKRYMAP EVLDESLNKN
421  HFQPYIMADI YSFGLIIWEM ARRCITGGIV EEYQLPYYNM VPSDPSYEDM REVVCVKRLR
481  PIVSNRWNSD ECLRAVLKLM SECWAHNPAS RLTALRIKKT LAKMVESQDV KI
     (SEQ ID NO: 22)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK3 polypeptide sequence is as follows:

```
  1  QNLDSMLHGT GMKSDSDQKK SENGVTLAPE DTLPFLKCYC SGHCPDDAIN NTCITNGHCF
 61  AIIEEDDQGE TTLASGCMKY EGSDFQCKDS PKAQLRRTIE CCRTNLCNQY LQPTLPPVVI
121  GPFFDGSIR  (SEQ ID NO: 23)
```

A nucleic acid sequence encoding human ALK3 precursor protein is shown in SEQ ID NO: 24, corresponding to nucleotides 549-2144 of Genbank Reference Sequence NM_004329.2. The signal sequence is underlined and the extracellular domain is indicated

in **bold** font. A nucleic acid sequence encoding the extracelluar human ALK3 polypeptide is shown in SEQ ID NO: 25.

A general formula for an active (*e.g.*, ligand binding) ALK3 polypeptide is one that comprises a polypeptide that begins at any amino acid position 25-31 (*i.e.*, position 25, 26, 27, 28, 29, 30, or 31) of SEQ ID NO: 22 and ends at any amino acid position 140-152 of SEQ ID NO: 22 (*i.e.*, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, or 152). See U.S. Patent 8,338,377, the teachings of which are incorporated herein by reference in their entirety.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK3 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK3 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK3). In other preferred embodiments, ALK3 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises an amino acid beginning at any amino acid position 25-31 (*i.e.*, position 25, 26, 27, 28, 29, 30, or 31) of SEQ ID NO: 22 and ending at any amino acid position 140-153 of SEQ ID NO: 22 (*i.e.*, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, or 152) of SEQ ID NO: 22. In some embodiments, heteromultimer complexes of the disclosure comprise at least one ALK3 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 22, 23, 115, 117, 407, or 408. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one ALK3 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 22, 23, 115, 117, 407, or 408.

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK4 polypeptide. As used herein, the term "ALK4" refers to a family of activin receptor-like kinase-4 proteins from any species and variants derived from such ALK4 proteins by mutagenesis or other modification. Reference to ALK4 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK4 family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ALK4 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK4 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

A human ALK4 precursor protein sequence (NCBI Ref Seq NP_004293) is as follows:

```
  1 MAESAGASSF FPLVVLLLAG SGGSGPRGVQ ALLCACTSCL QANYTCETDG ACMVSIFNLD
 61 GMEHHVRTCI PKVELVPAGK PFYCLSSEDL RNTHCCYTDY CNRIDLRVPS GHLKEPEHPS
121 MWGPVELVGI IAGPVFLLFL IIIIVFLVIN YHQRVYHNRQ RLDMEDPSCE MCLSKDKTLQ
181 DLVYDLSTSG SGSGLPLFVQ RTVARTIVLQ EIIGKGRFGE VWRGRWRGGD VAVKIFSSRE
241 ERSWFREAEI YQTVMLRHEN ILGFIAADNK DNGTWTQLWL VSDYHEHGSL FDYLNRYTVT
301 IEGMIKLALS AASGLAHLHM EIVGTQGKPG IAHRDLKSKN ILVKKNGMCA IADLGLAVRH
361 DAVTDTIDIA PNQRVGTKRY MAPEVLDETI NMKHFDSFKC ADIYALGLVY WEIARRCNSG
421 GVHEEYQLPY YDLVPSDPSI EEMRKVVCDQ KLRPNIPNWW QSYEALRVMG KMMRECWYAN
481 GAARLTALRI KKTLSQLSVQ EDVKI (SEQ ID NO: 26)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular human ALK4 polypeptide sequence is as follows:

```
SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGKPFYCL
SSEDLRNTHCCYTDYCNRIDLRVPSGHLKEPEHPSMWGPVE (SEQ ID NO: 27)
```

A nucleic acid sequence encoding an ALK4 precursor protein is shown in SEQ ID NO: 28), corresponding to nucleotides 78-1592 of Genbank Reference Sequence NM_004302.4. A nucleic acid sequence encoding the extracellular ALK4 polypeptide is shown in SEQ ID NO: 28

An alternative isoform of human ALK4 precursor protein sequence, isoform C (NCBI Ref Seq NP_064733.3), is as follows:

```
  1 MAESAGASSF FPLVVLLLAG SGGSGPRGVQ ALLCACTSCL QANYTCETDG ACMVSIFNLD
 61 GMEHHVRTCI PKVELVPAGK PFYCLSSEDL RNTHCCYTDY CNRIDLRVPS GHLKEPEHPS
121 MWGPVELVGI IAGPVFLLFL IIIIVFLVIN YHQRVYHNRQ RLDMEDPSCE MCLSKDKTLQ
181 DLVYDLSTSG SGSGLPLFVQ RTVARTIVLQ EIIGKGRFGE VWRGRWRGGD VAVKIFSSRE
241 ERSWFREAEI YQTVMLRHEN ILGFIAADNK ADCSFLTLPW EVVMVSAAPK LRSLRLQYKG
301 GRGRARFLFP LNNGTWTQLW LVSDYHEHGS LFDYLNRYTV TIEGMIKLAL SAASGLAHLH
361 MEIVGTQGKP GIAHRDLKSK NILVKKNGMC AIADLGLAVR HDAVTDTIDI APNQRVGTKR
421 YMAPEVLDET INMKHFDSFK CADIYALGLV YWEIARRCNS GGVHEEYQLP YYDLVPSDPS
481 IEEMRKVVCD QKLRPNIPNW WQSYEALRVM GKMMRECWYA NGAARLTALR IKKTLSQLSV
541 QEDVKI (SEQ ID NO: 83)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK4 polypeptide sequence (isoform C) is as follows:

SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGKPFYCL
SSEDLRNTHCCYTDYCNRIDLRVPSGHLKEPEHPSMWGPVE (SEQ ID NO: 84)

A nucleic acid sequence encoding an ALK4 precursor protein (isoform C) is shown in SEQ ID NO: 85, corresponding to nucleotides 78-1715 of Genbank Reference Sequence NM_020328.3. A nucleic acid sequence encoding the extracelluar ALK4 polypeptide (isoform C) is shown in SEQ ID NO: 86.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK4 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK4 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK4). In other preferred embodiments, ALK4 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK4 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 26, 27, 83, 84, 104, 106, 403, or 404. In some embodiments, heteromultimers of the disclosure consist or consist essentially of at least one ALK4 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 26, 27, 83, 84, 104, 106, 403, or 404.

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK5 polypeptide. As used herein, the term "ALK5" refers to a family of activin receptor-like kinase-5 proteins from any species and variants derived from such ALK4 proteins by mutagenesis or other modification. Reference to ALK5 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK5 family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ALK5 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK5 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

A human ALK5 precursor protein sequence (NCBI Ref Seq NP_004603.1) is as follows:

```
  1 MEAAVAAPRP RLLLLVLAAA AAAAALLPG ATALQCFCHL CTKDNFTCVT DGLCFVSVTE
 61 TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE LPTTVKSSPG
121 LGPVELAAVI AGPVCFVCIS LMLMVYICHN RTVIHHRVPN EEDPSLDRPF ISEGTTLKDL
181 IYDMTTSGSG SGLPLLVQRT IARTIVLQES IGKGRFGEVW RGKWRGEEVA VKIFSSREER
241 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS DYHEHGSLFD YLNRYTVTVE
301 GMIKLALSTA SGLAHLHMEI VGTQGKPAIA HRDLKSKNIL VKKNGTCCIA DLGLAVRHDS
361 ATDTIDIAPN HRVGTKRYMA PEVLDDSINM KHFESFKRAD IYAMGLVFWE IARRCSIGGI
421 HEDYQLPYYD LVPSDPSVEE MRKVVCEQKL RPNIPNRWQS CEALRVMAKI MRECWYANGA
481 ARLTALRIKK TLSQLSQQEG IKM (SEQ ID NO: 30)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK5 polypeptide sequence is as follows:

```
AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVC
APSSKTGSVTTTYCCNQDHCNKIELPTTVKSSPGLGPVEL (SEQ ID NO: 31)
```

A nucleic acid sequence encoding the ALK5 precursor protein is shown in SEQ ID NO: 32, corresponding to nucleotides 77-1585 of Genbank Reference Sequence NM_004612.2. A nucleic acid sequence encoding an extracellular human ALK5 polypeptide is shown in SEQ ID NO: 33.

An alternative isoform of the human ALK5 precursor protein sequence, isoform 2 (NCBI Ref Seq XP_005252207.1), is as follows:

```
  1 MEAAVAAPRP RLLLLVLAAA AAAAALLPG ATALQCFCHL CTKDNFTCVT DGLCFVSVTE
 61 TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE LPTTGPFSVK
121 SSPGLGPVEL AAVIAGPVCF VCISLMLMVY ICHNRTVIHH RVPNEEDPSL DRPFISEGTT
181 LKDLIYDMTT SGSGSGLPLL VQRTIARTIV LQESIGKGRF GEVWRGKWRG EEVAVKIFSS
241 REERSWFREA EIYQTVMLRH ENILGFIAAD NKDNGTWTQL WLVSDYHEHG SLFDYLNRYT
301 VTVEGMIKLA LSTASGLAHL HMEIVGTQGK PAIAHRDLKS KNILVKKNGT CCIADLGLAV
361 RHDSATDTID IAPNHRVGTK RYMAPEVLDD SINMKHFESF KRADIYAMGL VFWEIARRCS
421 IGGIHEDYQL PYYDLVPSDP SVEEMRKVVC EQKLRPNIPN RWQSCEALRV MAKIMRECWY
481 ANGAARLTAL RIKKTLSQLS QQEGIKM (SEQ ID NO: 87)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK5 polypeptide sequence (isoform 2) is as follows:

AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVC
APSSKTGSVTTTYCCNQDHCNKIELPTTGPFSVKSSPGLGPVEL (SEQ ID NO: 88)

A nucleic acid sequence encoding human ALK5 precursor protein (isoform 2) is shown in SEQ ID NO: 89, corresponding to nucleotides 77-1597 of Genbank Reference Sequence XM_005252150.1. A nucleic acid sequence encoding a processed extracellular ALK5 polypeptide is shown in SEQ ID NO: 90.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK5 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK5 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK5). In other preferred embodiments, ALK5 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 30, 31, 87, or 88. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 30, 31, 87, or 88.

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK6 polypeptide. As used herein, the term "ALK6" refers to a family of activin receptor-like kinase-6 proteins from any species and variants derived from such ALK6 proteins by mutagenesis or other modification. Reference to ALK6 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK6 family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ALK6 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK6 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

A human ALK6 precursor protein sequence (NCBI Ref Seq NP_001194.1) is as follows:

```
  1 MLLRSAGKLN VGTKKEDGES TAPTPRPKVL RCKCHHHCPE DSVNNICSTD GYCFTMIEED
 61 DSGLPVVTSG CLGLEGSDFQ CRDTPIPHQR RSIECCTERN ECNKDLHPTL PPLKNRDFVD
121 GPIHHRALLI SVTVCSLLLV LIILFCYFRY KRQETRPRYS IGLEQDETYI PPGESLRDLI
181 EQSQSSGSGS GLPLLVQRTI AKQIQMVKQI GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS
241 WFRETEIYQT VLMRHENILG FIAADIKGTG SWTQLYLITD YHENGSLYDY LKSTTLDAKS
301 MLKLAYSSVS GLCHLHTEIF STQGKPAIAH RDLKSKNILV KKNGTCCIAD LGLAVKFISD
361 TNEVDIPPNT RVGTKRYMPP EVLDESLNRN HFQSYIMADM YSFGLILWEV ARRCVSGGIV
421 EEYQLPYHDL VPSDPSYEDM REIVCIKKLR PSFPNRWSSD ECLRQMGKLM TECWAHNPAS
481 RLTALRVKKT LAKMSESQDI KL (SEQ ID NO: 34)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

The processed extracellular ALK6 polypeptide sequence is as follows:

```
KKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMIEEDDSGLPVVTSGCLGLE
GSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFVDGPIHHR (SEQ ID
NO: 35)
```

A nucleic acid sequence encoding the ALK6 precursor protein is shown in SEQ ID NO: 36, corresponding to nucleotides 275-1780 of Genbank Reference Sequence NM_001203.2. A nucleic acid sequence encoding processed extracellular ALK6 polypeptide is shown in SEQ ID NO: 37.

An alternative isoform of human ALK6 precursor protein sequence, isoform 2 (NCBI Ref Seq NP_001243722.1) is as follows:

```
  1 MGWLEELNWQ LHIFLLILLS MHTRANFLDN MLLRSAGKLN VGTKKEDGES TAPTPRPKVL
 61 RCKCHHHCPE DSVNNICSTD GYCFTMIEED DSGLPVVTSG CLGLEGSDFQ CRDTPIPHQR
121 RSIECCTERN ECNKDLHPTL PPLKNRDFVD GPIHHRALLI SVTVCSLLLV LIILFCYFRY
181 KRQETRPRYS IGLEQDETYI PPGESLRDLI EQSQSSGSGS GLPLLVQRTI AKQIQMVKQI
241 GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT VLMRHENILG FIAADIKGTG
301 SWTQLYLITD YHENGSLYDY LKSTTLDAKS MLKLAYSSVS GLCHLHTEIF STQGKPAIAH
361 RDLKSKNILV KKNGTCCIAD LGLAVKFISD TNEVDIPPNT RVGTKRYMPP EVLDESLNRN
421 HFQSYIMADM YSFGLILWEV ARRCVSGGIV EEYQLPYHDL VPSDPSYEDM REIVCIKKLR
```

481 PSFPNRWSSD ECLRQMGKLM TECWAHNPAS RLTALRVKKT LAKMSESQDI KL (SEQ ID NO: 91)

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK6 polypeptide sequence (isoform 2) is as follows:

NFLDNMLLRSAGKLNVGTKKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMI EEDDSGLPVVTSGCLGLEGSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFV DGPIHHR (SEQ ID NO: 92)

A nucleic acid sequence encoding human ALK6 precursor protein (isoform 2) is shown in SEQ ID NO: 93, corresponding to nucleotides 22-1617 of Genbank Reference Sequence NM_001256793.1. A nucleic acid sequence encoding a processed extracellular ALK6 polypeptide is shown in SEQ ID NO: 94.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK6 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK6 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK6). In other preferred embodiments, ALK6 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK6 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 34, 35, 91, or 92. In some embodiments, heteromultimers of the disclosure consist or consist essentially of at least one ALK6 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 34, 35, 91, or 92.

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK7 polypeptide. As used herein, the term "ALK7" refers to a family of activin receptor-like kinase-7 proteins from any species and variants derived from such ALK7 proteins by mutagenesis or other modification. Reference to ALK7 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK7 family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-

rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ALK7 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK7 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

Four naturally occurring isoforms of human ALK7 have been described. The sequence of human ALK7 isoform 1 precursor protein (NCBI Ref Seq NP_660302.2) is as follows:

```
  1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI
 61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PMELAIIITV
121 PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL IYDVTASGSG
181 SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ
241 TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA
301 SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN
361 PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD
421 MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK
481 TISQLCVKED CKA (SEQ ID NO: 38)
```

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK7 isoform 1 polypeptide sequence is as follows:

ELSPGLKCVCLLCDSSNFTCQTEGACWASVMLTNGKEQVIKSCVSLPELNAQVFCHSSNNVT KTECCFTDFCNNITLHLPTASPNAPKLGPME (SEQ ID NO: 39)

A nucleic acid sequence encoding human ALK7 isoform 1 precursor protein is shown below in SEQ ID NO: 40, corresponding to nucleotides 244-1722 of Genbank Reference Sequence NM_145259.2. A nucleic acid sequence encoding the processed extracellular ALK7 polypeptide (isoform 1) is show in in SEQ ID NO: 41.

An amino acid sequence of an alternative isoform of human ALK7, isoform 2 (NCBI Ref Seq NP_001104501.1), is shown in its processed form as follows (SEQ ID NO: 301), where the extracellular domain is indicated in **bold** font.

```
  1 MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG
 61 PMELAIIITV PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL
121 IYDVTASGSG SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER
181 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA
```

241 GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS
301 ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI
361 VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA
421 ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 301)

An amino acid sequence of the extracellular ALK7 polypeptide (isoform 2) is as follows:

MLTNGKEQVIKSCVSLPELNAQVFCHSSNNVTKTECCFTDFCNNITLHLPTASPNAPKLGPME (SEQ ID NO: 302).

A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 2) is shown below in SEQ ID NO: 303, corresponding to nucleotides 279-1607 of NCBI Reference Sequence NM_001111031.1.

A nucleic acid sequence encoding an extracellular ALK7 polypeptide (isoform 2) is shown in SEQ ID NO: 304.

An amino acid sequence of an alternative human ALK7 precursor protein, isoform 3 (NCBI Ref Seq NP_001104502.1), is shown as follows (SEQ ID NO: 305), where the signal peptide is indicated by a single underline.

1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI
61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TGLPLLVQRT IARTIVLQEI
121 VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ TVMLRHENIL GFIAADNKDN
181 GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA SGLAHLHMEI VGTQGKPAIA
241 HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV
301 NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF
361 RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK TISQLCVKED CKA
(SEQ ID NO: 305)

The amino acid sequence of a processed ALK7 polypeptide (isoform 3) is as follows (SEQ ID NO: 306). This isoform lacks a transmembrane domain and is therefore proposed to be soluble in its entirety (Roberts et al., 2003, Biol Reprod 68:1719-1726). N-terminal variants of SEQ ID NO: 306 are predicted as described below.

1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN
61 VTKTECCFTD FCNNITLHLP TGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA
121 VKIFSSRDER SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD
181 YLNRNIVTVA GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA
241 DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE
301 IARRCSVGGI VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI
361 MRECWYANGA ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 306)

A nucleic acid sequence encoding an unprocessed ALK7 polypeptide precursor protein (isoform 3) is shown in SEQ ID NO: 307, corresponding to nucleotides 244-1482 of NCBI Reference Sequence NM_001111032.1. A nucleic acid sequence encoding a processed ALK7 polypeptide (isoform 3) is shown in SEQ ID NO: 308.

An amino acid sequence of an alternative human ALK7 precursor protein, isoform 4 (NCBI Ref Seq NP_001104503.1), is shown as follows (SEQ ID NO: 309), where the signal peptide is indicated by a single underline.

```
  1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI
 61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TDNGTWTQLW LVSEYHEQGS
121 LYDYLNRNIV TVAGMIKLAL SIASGLAHLH MEIVGTQGKP AIAHRDIKSK NILVKKCETC
181 AIADLGLAVK HDSILNTIDI PQNPKVGTKR YMAPEMLDDT MNVNIFESFK RADIYSVGLV
241 YWEIARRCSV GGIVEEYQLP YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM
301 GRIMRECWYA NGAARLTALR IKKTISQLCV KEDCKA  (SEQ ID NO: 309)
```

An amino acid sequence of a processed ALK7 polypeptide (isoform 4) is as follows (SEQ ID NO: 310). Like ALK7 isoform 3, isoform 4 lacks a transmembrane domain and is therefore proposed to be soluble in its entirety (Roberts et al., 2003, Biol Reprod 68:1719-1726). N-terminal variants of SEQ ID NO: 310 are predicted as described below.

```
  1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN
 61 VTKTECCFTD FCNNITLHLP TDNGTWTQLW LVSEYHEQGS LYDYLNRNIV TVAGMIKLAL
121 SIASGLAHLH MEIVGTQGKP AIAHRDIKSK NILVKKCETC AIADLGLAVK HDSILNTIDI
181 PQNPKVGTKR YMAPEMLDDT MNVNIFESFK RADIYSVGLV YWEIARRCSV GGIVEEYQLP
240 YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM GRIMRECWYA NGAARLTALR
301 IKKTISQLCV KEDCKA   (SEQ ID NO: 310)
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A nucleic acid sequence encoding the unprocessed ALK7 polypeptide precursor protein (isoform 4) is shown in SEQ ID NO: 311, corresponding to nucleotides 244-1244 of NCBI Reference Sequence NM_001111033.1. A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 4) is shown in SEQ ID NO: 312.

Based on the signal sequence of full-length ALK7 (isoform 1) in the rat (see NCBI Reference Sequence NP_620790.1) and on the high degree of sequence identity between human and rat ALK7, it is predicted that a processed form of human ALK7 isoform 1 is as follows (SEQ ID NO: 313).

```
  1 LKCVCLLCDS SNFTCQTEGA CWASVMLTNG KEQVIKSCVS LPELNAQVFC HSSNNVTKTE
 61 CCFTDFCNNI TLHLPTASPN APKLGPME          (SEQ ID NO: 313)
```

Active variants of processed ALK7 isoform 1 are predicted in which SEQ ID NO: 39 is truncated by 1, 2, 3, 4, 5, 6, or 7 amino acids at the N-terminus and SEQ ID NO: 313 is truncated by 1 or 2 amino acids at the N-terminus. Consistent with SEQ ID NO: 313, it is further expected that leucine is the N-terminal amino acid in the processed forms of human ALK7 isoform 3 (SEQ ID NO: 306) and human ALK7 isoform 4 (SEQ ID NO: 310).

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK7 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK7 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK7). In other preferred embodiments, ALK7 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK7 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 38, 39, 112, 114, 301, 302, 305, 306, 309, 310, 313, 405, or 406. In some embodiments, heteromultimers of the disclosure consist or consist essentially of at least one ALK7 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 38, 39, 112, 114, 301, 302, 305, 306, 309, 310, 313, 405, or 406.

In some embodiments, the present disclosure contemplates making functional variants by modifying the structure of a TGF-beta superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) and/or a TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII) for such purposes as enhancing therapeutic efficacy or stability (*e.g.*, shelf-life and resistance to proteolytic degradation *in vivo*). Variants can be produced by amino acid substitution, deletion, addition, or combinations thereof. For instance, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*e.g.*, conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of a polypeptide of the disclosure results in a functional homolog can be

readily determined by assessing the ability of the variant polypeptide to produce a response in cells in a fashion similar to the wild-type polypeptide, or to bind to one or more TGF-beta superfamily ligands including, for example, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty.

In some embodiments, the present disclosure contemplates making functional variants by modifying the structure of the TGF-beta superfamily type I receptor polypeptide and/or TGF-beta superfamily type II receptor polypeptide for such purposes as enhancing therapeutic efficacy or stability (e.g., increased shelf-life and/or increased resistance to proteolytic degradation).

In certain embodiments, the present disclosure contemplates specific mutations of a TGF-beta superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) and/or a TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII) receptor of the disclosure so as to alter the glycosylation of the polypeptide. Such mutations may be selected so as to introduce or eliminate one or more glycosylation sites, such as O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine or asparagine-X-serine (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. Removal of one or more carbohydrate moieties present

on a polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of a polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* [Meth. Enzymol. (1987) 138:350]. The sequence of a polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect, and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, TGF-beta superfamily type I and II receptor complexes of the present disclosure for use in humans may be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other mammalian expression cell lines are expected to be useful as well.

The present disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of a TGF-beta superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) and/or a TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII) disclosed herein, as well as truncation mutants. Pools of combinatorial mutants are especially useful for identifying functionally active (e.g., ligand binding) TGF-beta superfamily type I and/or TGF-beta superfamily type II receptor sequences. The purpose of screening such combinatorial libraries may be to generate, for example, polypeptides variants which have altered properties, such as altered pharmacokinetic or altered ligand binding. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, TGF-beta superfamily type I and II receptor complex variants may be screened for ability to bind to a TGF-beta superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty), to prevent binding of a TGF-beta superfamily ligand to a TGF-beta superfamily receptor, and/or to interfere with signaling caused by an TGF-beta superfamily ligand.

The activity of a TGF-beta superfamily heteromultimer of the disclosure also may be tested, for example in a cell-based or *in vivo* assay. For example, the effect of a heteromultimer complex on the expression of genes or the activity of proteins involved in muscle production in a muscle cell may be assessed. This may, as needed, be performed in the presence of one or more recombinant TGF-beta superfamily ligand proteins (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty), and cells may be transfected so as to produce a TGF-beta superfamily type I and II receptor complex, and optionally, a TGF-beta superfamily ligand. Likewise, a heteromultimer complex of the disclosure may be administered to a mouse or other animal, and one or more measurements, such as muscle formation and strength may be assessed using art-recognized methods. Similarly, the activity of a heteromultimer, or variants thereof, may be tested in osteoblasts, adipocytes, and/or neuronal cells for any effect on growth of these cells, for example, by the assays as described herein and those of common knowledge in the art. A SMAD-responsive reporter gene may be used in such cell lines to monitor effects on downstream signaling.

Combinatorial-derived variants can be generated which have increased selectivity or generally increased potency relative to a reference TGF-beta superfamily heteromultimer. Such variants, when expressed from recombinant DNA constructs, can be used in gene therapy protocols. Likewise, mutagenesis can give rise to variants which have intracellular half-lives dramatically different than the corresponding unmodified TGF-beta superfamily heteromultimer. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other cellular processes which result in destruction, or otherwise inactivation, of an unmodified polypeptide. Such variants, and the genes which encode them, can be utilized to alter polypeptide complex levels by modulating the half-life of the polypeptide. For instance, a short half-life can give rise to more transient biological effects and, when part of an inducible expression system, can allow tighter control of recombinant polypeptide complex levels within the cell. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter one or more activities of the TGF-beta superfamily heteromultimer complex including, for example, immunogenicity, half-life, and solubility.

A combinatorial library may be produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential TGF-beta superfamily type I and/or II receptor sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential TGF-beta superfamily type I and/or II receptor encoding nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display).

There are many ways by which the library of potential homologs can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes can then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art. See, *e.g.*, Narang, SA (1983) Tetrahedron 39:3; Itakura *et al.* (1981) Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura *et al.* (1984) Annu. Rev. Biochem. 53:323; Itakura *et al.* (1984) Science 198:1056; Ike *et al.* (1983) Nucleic Acid Res. 11:477. Such techniques have been employed in the directed evolution of other proteins. See, *e.g.*, Scott *et al.*, (1990) Science 249:386-390; Roberts *et al.* (1992) PNAS USA 89:2429-2433; Devlin *et al.* (1990) Science 249: 404-406; Cwirla *et al.*, (1990) PNAS USA 87: 6378-6382; as well as U.S. Patent Nos: 5,223,409, 5,198,346, and 5,096,815.

Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, TGF-beta superfamily heteromultimers of the disclosure can be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis [see, *e.g.*, Ruf *et al.* (1994) Biochemistry 33:1565-1572; Wang *et al.* (1994) J. Biol. Chem. 269:3095-3099; Balint *et al.* (1993) Gene 137:109-118; Grodberg *et al.* (1993) Eur. J. Biochem. 218:597-601; Nagashima *et al.* (1993) J. Biol. Chem. 268:2888-2892; Lowman *et al.* (1991) Biochemistry 30:10832-10838; and Cunningham *et al.* (1989) Science 244:1081-1085], by linker scanning mutagenesis [see, *e.g.*, Gustin *et al.* (1993) Virology 193:653-660; and Brown *et al.* (1992) Mol. Cell Biol. 12:2644-2652; McKnight *et al.* (1982) Science 232:316], by saturation mutagenesis [see, *e.g.*, Meyers *et al.*, (1986) Science 232:613]; by PCR mutagenesis [see, *e.g.*, Leung *et al.* (1989) Method Cell Mol Biol 1:11-19]; or by random mutagenesis, including chemical mutagenesis [*see*, *e.g.*, Miller *et al.* (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, NY; and Greener *et al.* (1994) Strategies in Mol Biol 7:32-34]. Linker scanning mutagenesis, particularly in a

combinatorial setting, is an attractive method for identifying truncated (bioactive) forms of TGF-beta superfamily type I and/or II receptor polypeptides.

A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and, for that matter, for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TGF-beta superfamily heteromultimers of the disclosure. The most widely used techniques for screening large gene libraries typically comprise cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include TGF-beta superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty) binding assays and/or TGF-beta superfamily ligand-mediated cell signaling assays.

In certain embodiments, TGF-beta superfamily type I and II heteromultimers of the disclosure may further comprise post-translational modifications in addition to any that are naturally present in the TGF-beta superfamily type I and/or II receptor polypeptide. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, the TGF-beta superfamily type I and II heteromultimer may comprise non-amino acid elements, such as polyethylene glycols, lipids, polysaccharide or monosaccharide, and phosphates. Effects of such non-amino acid elements on the functionality of a heteromultimer complex may be tested as described herein for other heteromultimer complex variants. When a polypeptide of the disclosure is produced in cells by cleaving a nascent form of the polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (*e.g.*, CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the TGF-beta superfamily type I and/or type II receptor polypeptides as well as heteromultimers comprising the same.

In certain aspects, the polypeptides disclosed herein may form protein complexes comprising at least one TGF-beta superfamily type I polypeptide associated, covalently or non-covalently, with at least one type II receptor polypeptide. Preferably, polypeptides disclosed herein form heterodimers, although higher order heteromultimers are also included such as, but not limited to, heterotrimers, heterotetramers, and further oligomeric structures (see, e.g., Figures 1, 2, and 15). In some embodiments, TGF-beta superfamily type I and/or type II receptor polypeptides of the present disclosure comprise at least one multimerization domain. As disclosed herein, the term "multimerization domain" refers to an amino acid or sequence of amino acids that promote covalent or non-covalent interaction between at least a first polypeptide and at least a second polypeptide. Polypeptides disclosed herein may be joined covalently or non-covalently to a multimerization domain. Preferably, a multimerization domain promotes interaction between a first polypeptide (*e.g.*, TGF-beta superfamily type I polypeptide) and a second polypeptide (*e.g.*, TGF-beta superfamily type II polypeptide) to promote heteromultimer formation (*e.g.*, heterodimer formation), and optionally hinders or otherwise disfavors homomultimer formation (*e.g.*, homodimer formation), thereby increasing the yield of desired heteromultimer (see, e.g., Figure 1, 2, or 15).

Many methods known in the art can be used to generate TGF-beta superfamily heteromultimers of the disclosure. For example, non-naturally occurring disulfide bonds may be constructed by replacing on a first polypeptide (*e.g.*, TGF-beta superfamily type I polypeptide) a naturally occurring amino acid with a free thiol-containing residue, such as cysteine, such that the free thiol interacts with another free thiol-containing residue on a second polypeptide (*e.g.*, TGF-beta superfamily type II polypeptide) such that a disulfide bond is formed between the first and second polypeptides. Additional examples of interactions to promote heteromultimer formation include, but are not limited to, ionic interactions such as described in Kjaergaard *et al.*, WO2007147901; electrostatic steering effects such as described in Kannan *et al.*, U.S.8,592,562; coiled-coil interactions such as described in Christensen *et al.*, U.S.20120302737; leucine zippers such as described in Pack & Plueckthun,(1992) Biochemistry 31: 1579-1584; and helix-turn-helix motifs such as described in Pack *et al.*, (1993) Bio/Technology 11: 1271-1277. Linkage of the various segments may be obtained via, *e.g.*, covalent binding such as by chemical cross-linking, peptide linkers, disulfide bridges, *etc.*, or affinity interactions such as by avidin-biotin or leucine zipper technology.

In certain aspects, a multimerization domain may comprise one component of an interaction pair. In some embodiments, the polypeptides disclosed herein may form protein complexes comprising a first polypeptide covalently or non-covalently associated with a second polypeptide, wherein the first polypeptide comprises the amino acid sequence of a TGF-beta superfamily type I polypeptide and the amino acid sequence of a first member of an interaction pair; and the second polypeptide comprises the amino acid sequence of a TGF-beta superfamily type II polypeptide and the amino acid sequence of a second member of an interaction pair. The interaction pair may be any two polypeptide sequences that interact to form a complex, particularly a heterodimeric complex although operative embodiments may also employ an interaction pair that can form a homodimeric complex. One member of the interaction pair may be fused to a TGF-beta superfamily type I or type II polypeptide as described herein, including for example, a polypeptide sequence comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of any one of SEQ ID NOs: 14, 15, 124, 126, 413, 414, 18, 19, 136, 138, 421, 422, 22, 23, 115, 117, 407, 408, 26, 27, 83, 84, 104, 106, 403, 404, 30, 31, 87, 88, 139, 141, 423, 424, 34, 35, 91, 92, 142, 144, 425, 426, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 405, 406, 9, 10, 11, 118, 120, 409, 410, 1, 2, 3, 4, 5, 6, 100, 102, 401, 402, 46, 47, 71, 72, 121, 123, 411, 412, 50, 51, 75, 76, 79, 80, 42, 43, 67, 68, 127, 129, 130, 132, 415, 416, 417, and 418. An interaction pair may be selected to confer an improved property/activity such as increased serum half-life, or to act as an adaptor on to which another moiety is attached to provide an improved property/activity. For example, a polyethylene glycol moiety may be attached to one or both components of an interaction pair to provide an improved property/activity such as improved serum half-life.

The first and second members of the interaction pair may be an asymmetric pair, meaning that the members of the pair preferentially associate with each other rather than self-associate. Accordingly, first and second members of an asymmetric interaction pair may associate to form a heterodimeric complex (see, e.g., Figures 2 and 15). Alternatively, the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and thus may have the same or different amino acid sequences. Accordingly, first and second members of an unguided interaction pair may associate to form a homodimer complex or a heterodimeric complex. Optionally, the first member of the interaction pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates covalently with the second member of the interaction pair.

Optionally, the first member of the interaction pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates non-covalently with the second member of the interaction pair.

As specific examples, the present disclosure provides fusion proteins comprising TGF-beta superfamily type I or type II polypeptides fused to a polypeptide comprising a constant domain of an immunoglobulin, such as a CH1, CH2, or CH3 domain of an immunoglobulin or an Fc domain. Fc domains derived from human IgG1, IgG2, IgG3, and IgG4 are provided herein. Other mutations are known that decrease either CDC or ADCC activity, and collectively, any of these variants are included in the disclosure and may be used as advantageous components of a heteromultimeric complex of the disclosure. Optionally, the IgG1 Fc domain of SEQ ID NO: 208 has one or more mutations at residues such as Asp-265, Lys-322, and Asn-434 (numbered in accordance with the corresponding full-length IgG1). In certain cases, the mutant Fc domain having one or more of these mutations (e.g., Asp-265 mutation) has reduced ability of binding to the Fcγ receptor relative to a wildtype Fc domain. In other cases, the mutant Fc domain having one or more of these mutations (e.g., Asn-434 mutation) has increased ability of binding to the MHC class I-related Fc-receptor (FcRN) relative to a wildtype Fc domain.

An example of a native amino acid sequence that may be used for the Fc portion of human IgG1 (G1Fc) is shown below (SEQ ID NO: 3100). Dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants. In part, the disclosure provides polypeptides comprising, consisting of, or consisting essentially of an amino acid sequence with 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 3100. Naturally occurring variants in G1Fc would include E134D and M136L according to the numbering system used in SEQ ID NO: 3100 (see Uniprot P01857).

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  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 3100)
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An example of a native amino acid sequence that may be used for the Fc portion of human IgG2 (G2Fc) is shown below (SEQ ID NO: 3200). Dotted underline indicates the hinge region and double underline indicates positions where there are data base conflicts in the sequence (according to UniProt P01859). In part, the disclosure provides polypeptides

comprising, consisting of, or consisting essentially of an amino acid sequence with 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 3200.

```
  1  VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
 51  FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS
101  NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
151  SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS
201  CSVMHEALHN HYTQKSLSLS PGK          (SEQ ID NO: 3200)
```

Two examples of amino acid sequences that may be used for the Fc portion of human IgG3 (G3Fc) are shown below. The hinge region in G3Fc can be up to four times as long as in other Fc chains and contains three identical 15-residue segments preceded by a similar 17-residue segment. The first G3Fc sequence shown below (SEQ ID NO: 3300) contains a short hinge region consisting of a single 15-residue segment, whereas the second G3Fc sequence (SEQ ID NO: 3400) contains a full-length hinge region. In each case, dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants according to UniProt P01859. In part, the disclosure provides polypeptides comprising, consisting of, or consisting essentially of an amino acid sequence with 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NOs: 3300 and 3400.

```
  1  EPKSCDTPPP CPRCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 51  VSHEDPEVQF KWYVDGVEVH NAKTKPREEQ YNSTFRVVSV LTVLHQDWLN
101  GKEYKCKVSN KALPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
151  TCLVKGFYPS DIAVEWESSG QPENNYNTTP PMLDSDGSFF LYSKLTVDKS
201  RWQQGNIFSC SVMHEALHNR FTQKSLSLSP GK     (SEQ ID NO: 3300)

  1  ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
 51  SCDTPPPCPR CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
101  EDPEVQFKWY VDGVEVHNAK TKPREEQYNS TFRVVSVLTV LHQDWLNGKE
151  YKCKVSNKAL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL
201  VKGFYPSDIA VEWESSGQPE NNYNTTPPML DSDGSFFLYS KLTVDKSRWQ
251  QGNIFSCSVM HEALHNRFTQ KSLSLSPGK        (SEQ ID NO: 3400)
```

Naturally occurring variants in G3Fc (for example, see Uniprot P01860) include E68Q, P76L, E79Q, Y81F, D97N, N100D, T124A, S169N, S169del, F221Y when converted to the numbering system used in SEQ ID NO: 3300, and the present disclosure provides

fusion proteins comprising G3Fc domains containing one or more of these variations. In addition, the human immunoglobulin IgG3 gene (*IGHG3*) shows a structural polymorphism characterized by different hinge lengths [see Uniprot P01859]. Specifically, variant WIS is lacking most of the V region and all of the CH1 region. It has an extra interchain disulfide bond at position 7 in addition to the 11 normally present in the hinge region. Variant ZUC lacks most of the V region, all of the CH1 region, and part of the hinge. Variant OMM may represent an allelic form or another gamma chain subclass. The present disclosure provides additional fusion proteins comprising G3Fc domains containing one or more of these variants.

An example of a native amino acid sequence that may be used for the Fc portion of human IgG4 (G4Fc) is shown below (SEQ ID NO: 3500). Dotted underline indicates the hinge region. In part, the disclosure provides polypeptides comprising, consisting of, or consisting essentially of an amino acid sequence with 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 3500.

```
  1  ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ
 51  EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE
101  YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL
151  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ
201  EGNVFSCSVM HEALHNHYTQ KSLSLSLGK      (SEQ ID NO: 3500)
```

A variety of engineered mutations in the Fc domain are presented herein with respect to the G1Fc sequence (SEQ ID NO: 3100), and analogous mutations in G2Fc, G3Fc, and G4Fc can be derived from their alignment with G1Fc in Figure 5. Due to unequal hinge lengths, analogous Fc positions based on isotype alignment (Figure 5) possess different amino acid numbers in SEQ ID NOs: 3100, 3200, 3300, and 3500. It can also be appreciated that a given amino acid position in an immunoglobulin sequence consisting of hinge, C_H2, and C_H3 regions (*e.g.*, SEQ ID NOs: 3100, 3200, 3300, and 3500) will be identified by a different number than the same position when numbering encompasses the entire IgG1 heavy-chain constant domain (consisting of the C_H1, hinge, C_H2, and C_H3 regions) as in the Uniprot database. For example, correspondence between selected C_H3 positions in a human G1Fc sequence (SEQ ID NO: 3100), the human IgG1 heavy chain constant domain (Uniprot P01857), and the human IgG1 heavy chain is as follows.

Correspondence of C_H3 Positions in Different Numbering Systems

G1Fc (Numbering begins at first threonine in hinge region)	IgG1 heavy chain constant domain (Numbering begins at C_H1)	IgG1 heavy chain (EU numbering scheme of Kabat et al., 1991*)
Y127	Y232	Y349
S132	S237	S354
E134	E239	E356
K138	K243	K360
T144	T249	T366
L146	L251	L368
N162	N267	N384
K170	K275	K392
D177	D282	D399
D179	D284	D401
Y185	Y290	Y407
K187	K292	K409
H213	H318	H435
K217	K322	K439
* Kabat et al. (eds) 1991; pp. 688-696 in *Sequences of Proteins of Immunological Interest*, 5th ed., Vol. 1, NIH, Bethesda, MD.		

A problem that arises in large-scale production of asymmetric immunoglobulin-based proteins from a single cell line is known as the "chain association issue". As confronted prominently in the production of bispecific antibodies, the chain association issue concerns the challenge of efficiently producing a desired multichain protein from among the multiple combinations that inherently result when different heavy chains and/or light chains are produced in a single cell line [see, for example, Klein et al (2012) mAbs 4:653-663]. This problem is most acute when two different heavy chains and two different light chains are produced in the same cell, in which case there are a total of 16 possible chain combinations (although some of these are identical) when only one is typically desired. Nevertheless, the same principle accounts for diminished yield of a desired multichain fusion protein that incorporates only two different (asymmetric) heavy chains.

Various methods are known in the art that increase desired pairing of Fc-containing fusion polypeptide chains in a single cell line to produce a preferred asymmetric fusion

protein at acceptable yields [see, for example, Klein et al (2012) mAbs 4:653-663; and Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Methods to obtain desired pairing of Fc-containing chains include, but are not limited to, charge-based pairing (electrostatic steering), "knobs-into-holes" steric pairing, SEEDbody pairing, and leucine zipper-based pairing. See, for example, Ridgway et al (1996) Protein Eng 9:617-621; Merchant et al (1998) Nat Biotech 16:677-681; Davis et al (2010) Protein Eng Des Sel 23:195-202; Gunasekaran et al (2010); 285:19637-19646; Wranik et al (2012) J Biol Chem 287:43331-43339; US5932448; WO 1993/011162; WO 2009/089004, and WO 2011/034605. As described herein, these methods may be used to generate heterodimers comprising TGF-beta superfamily type I and type II receptor polypeptides, at least two different TGF-beta superfamily type I receptor polypeptides, and at least two different TGF-beta superfamily type II receptor polypeptides. See Figure 15.

For example, one means by which interaction between specific polypeptides may be promoted is by engineering protuberance-into-cavity (knob-into-holes) complementary regions such as described in Arathoon *et al.*, U.S.7,183,076 and Carter *et al.*, U.S.5,731,168. "Protuberances" are constructed by replacing small amino acid side chains from the interface of the first polypeptide (*e.g.*, a first interaction pair) with larger side chains (*e.g.*, tyrosine or tryptophan). Complementary "cavities" of identical or similar size to the protuberances are optionally created on the interface of the second polypeptide (*e.g.*, a second interaction pair) by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine). Where a suitably positioned and dimensioned protuberance or cavity exists at the interface of either the first or second polypeptide, it is only necessary to engineer a corresponding cavity or protuberance, respectively, at the adjacent interface.

At neutral pH (7.0), aspartic acid and glutamic acid are negatively charged and lysine, arginine, and histidine are positively charged. These charged residues can be used to promote heterodimer formation and at the same time hinder homodimer formation. Attractive interactions take place between opposite charges and repulsive interactions occur between like charges. In part, protein complexes disclosed herein make use of the attractive interactions for promoting heteromultimer formation (*e.g.*, heterodimer formation), and optionally repulsive interactions for hindering homodimer formation (*e.g.*, homodimer formation) by carrying out site directed mutagenesis of charged interface residues.

For example, the IgG1 CH3 domain interface comprises four unique charge residue pairs involved in domain-domain interactions: Asp356-Lys439', Glu357-Lys370', Lys392-Asp399', and Asp399-Lys409' [residue numbering in the second chain is indicated by (')]. It should be noted that the numbering scheme used here to designate residues in the IgG1 CH3 domain conforms to the EU numbering scheme of Kabat. Due to the 2-fold symmetry present in the CH3-CH3 domain interactions, each unique interaction will represented twice in the structure (*e.g.*, Asp-399-Lys409' and Lys409-Asp399'). In the wild-type sequence, K409-D399' favors both heterodimer and homodimer formation. A single mutation switching the charge polarity (*e.g.*, K409E; positive to negative charge) in the first chain leads to unfavorable interactions for the formation of the first chain homodimer. The unfavorable interactions arise due to the repulsive interactions occurring between the same charges (negative-negative; K409E-D399' and D399-K409E'). A similar mutation switching the charge polarity (D399K'; negative to positive) in the second chain leads to unfavorable interactions (K409'-D399K' and D399K-K409') for the second chain homodimer formation. But, at the same time, these two mutations (K409E and D399K') lead to favorable interactions (K409E-D399K' and D399-K409') for the heterodimer formation.

The electrostatic steering effect on heterodimer formation and homodimer discouragement can be further enhanced by mutation of additional charge residues which may or may not be paired with an oppositely charged residue in the second chain including, for example, Arg355 and Lys360. The table below lists possible charge change mutations that can be used, alone or in combination, to enhance heteromultimer formation of the polypeptide complexes disclosed herein.

Examples of Pair-Wise Charged Residue Mutations to Enhance Heterodimer Formation			
Position in first chain	Mutation in first chain	Interacting position in second chain	Corresponding mutation in second chain
Lys409	Asp or Glu	Asp399'	Lys, Arg, or His
Lys392	Asp or Glu	Asp399'	Lys, Arg, or His
Lys439	Asp or Glu	Asp356'	Lys, Arg, or His
Lys370	Asp or Glu	Glu357'	Lys, Arg, or His
Asp399	Lys, Arg, or His	Lys409'	Asp or Glu
Asp399	Lys, Arg, or His	Lys392'	Asp or Glu
Asp356	Lys, Arg, or His	Lys439'	Asp or Glu

Glu357	Lys, Arg, or His	Lys370'	Asp or Glu

In some embodiments, one or more residues that make up the CH3-CH3 interface in a fusion protein of the instant application are replaced with a charged amino acid such that the interaction becomes electrostatically unfavorable. For example, a positive-charged amino acid in the interface (*e.g.*, a lysine, arginine, or histidine) is replaced with a negatively charged amino acid (*e.g.*, aspartic acid or glutamic acid). Alternatively, or in combination with the forgoing substitution, a negative-charged amino acid in the interface is replaced with a positive-charged amino acid. In certain embodiments, the amino acid is replaced with a non-naturally occurring amino acid having the desired charge characteristic. It should be noted that mutating negatively charged residues (Asp or Glu) to His will lead to increase in side chain volume, which may cause steric issues. Furthermore, His proton donor- and acceptor-form depends on the localized environment. These issues should be taken into consideration with the design strategy. Because the interface residues are highly conserved in human and mouse IgG subclasses, electrostatic steering effects disclosed herein can be applied to human and mouse IgG1, IgG2, IgG3, and IgG4. This strategy can also be extended to modifying uncharged residues to charged residues at the CH3 domain interface.

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered to be complementary on the basis of charge pairing (electrostatic steering). One of a pair of Fc sequences with electrostatic complementarity can be arbitrarily fused to the TGF-beta superfamily type I or type II receptor polypeptide of the construct, with or without an optional linker, to generate a TGF-beta superfamily type I or type II receptor fusion polypeptide This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc to favor generation of the desired multichain construct (e.g., a TGF-beta superfamily heteromeric complex). In this example based on electrostatic steering, SEQ ID NO: 200 [human G1Fc(E134K/D177K)] and SEQ ID NO: 201 [human G1Fc(K170D/K187D)] are examples of complementary Fc sequences in which the engineered amino acid substitutions are double underlined, and the TGF-beta superfamily type I or type II receptor polypeptide of the construct can be fused to either SEQ ID NO: 200 or SEQ ID NO: 201, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate

complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 200 and 201).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLKSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK        (SEQ ID NO: 200)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYD TTPPVLDSDG SFFLYSDLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK        (SEQ ID NO: 201)
```

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered for steric complementarity. In part, the disclosure provides knobs-into-holes pairing as an example of steric complementarity. One of a pair of Fc sequences with steric complementarity can be arbitrarily fused to the TGF-beta superfamily type I or type II polypeptide of the construct, with or without an optional linker, to generate a TGF-beta superfamily type I or type II fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc to favor generation of the desired multichain construct. In this example based on knobs-into-holes pairing, SEQ ID NO: 202 [human G1Fc(T144Y)] and SEQ ID NO: 203 [human G1Fc(Y185T)] are examples of complementary Fc sequences in which the engineered amino acid substitutions are double underlined, and the TGF-beta superfamily type I or type II polypeptide of the construct can be fused to either SEQ ID NO: 202 or SEQ ID NO: 203, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 202 and 203).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLYCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 202)
```

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLTSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 203)
```

An example of Fc complementarity based on knobs-into-holes pairing combined with an engineered disulfide bond is disclosed in SEQ ID NO: 204 [hG1Fc(S132C/T144W)] and SEQ ID NO: 205 [hG1Fc(Y127C/T144S/L146A/Y185V)]. The engineered amino acid substitutions in these sequences are double underlined, and the TGF-beta superfamily type I or type II polypeptide of the construct can be fused to either SEQ ID NO: 204 or SEQ ID NO: 205, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 204 and 205).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 204)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSREEMTKNQ VSLSCAVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 205)
```

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered to generate interdigitating β-strand segments of human IgG and IgA C_H3 domains. Such methods include the use of strand-exchange engineered domain (SEED) C_H3 heterodimers allowing the formation of SEEDbody fusion proteins [see, for example, Davis et al (2010) Protein Eng Design Sel 23:195-202]. One of a pair of Fc sequences with SEEDbody complementarity can be arbitrarily fused to the TGF-beta superfamily type I or type II polypeptide of the construct, with or without an optional linker, to generate a TGF-beta superfamily type I or type II fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc to favor generation of the

desired multichain construct. In this example based on SEEDbody (Sb) pairing, SEQ ID NO: 206 [hG1Fc(Sb_{AG})] and SEQ ID NO: 207 [hG1Fc(Sb_{GA})] are examples of complementary IgG Fc sequences in which the engineered amino acid substitutions from IgA Fc are double underlined, and the TGF-beta superfamily type I or type II polypeptide of the construct can be fused to either SEQ ID NO: 206 or SEQ ID NO: 207, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG1Fc, hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate an Fc monomer which may be used in the complementary IgG-IgA pair below (SEQ ID NOs: 206 and 207).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PFRPEVHLLP PSREEMTKNQ VSLTCLARGF
151  YPKDIAVEWE SNGQPENNYK TTPSRQEPSQ GTTTFAVTSK LTVDKSRWQQ
201  GNVFSCSVMH EALHNHYTQK TISLSPGK      (SEQ ID NO: 206)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PPSEELALNE LVTLTCLVKG
151  FYPSDIAVEW ESNGQELPRE KYLTWAPVLD SDGSFFLYSI LRVAAEDWKK
201  GDTFSCSVMH EALHNHYTQK SLDRSPGK      (SEQ ID NO: 207)
```

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains with a cleavable leucine zipper domain attached at the C-terminus of the Fc C_H3 domains. Attachment of a leucine zipper is sufficient to cause preferential assembly of heterodimeric antibody heavy chains. See, e.g., Wranik et al (2012) J Biol Chem 287:43331-43339. As disclosed herein, one of a pair of Fc sequences attached to a leucine zipper-forming strand can be arbitrarily fused to the TGF-beta superfamily type I or type II polypeptide of the construct, with or without an optional linker, to generate a TGF-beta superfamily type I or type II fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence attached to a complementary leucine zipper-forming strand to favor generation of the desired multichain construct. Proteolytic digestion of the construct with the bacterial endoproteinase Lys-C post purification can release the leucine zipper domain, resulting in an Fc construct whose structure is identical to that of native Fc. In this example based on leucine zipper pairing, SEQ ID NO: 213 [hG1Fc-Ap1 (acidic)] and SEQ ID NO: 214 [hG1Fc-Bp1 (basic)] are examples of complementary IgG Fc sequences in which the engineered complimentary leucine zipper sequences are underlined,

and the TGF-beta superfamily type I or type II polypeptide of the construct can be fused to either SEQ ID NO: 213 or SEQ ID NO: 214, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that leucine zipper-forming sequences attached, with or without an optional linker, to hG1Fc, hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate an Fc monomer which may be used in the complementary leucine zipper-forming pair below (SEQ ID NOs: 213 and 214).

```
  1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGKGGSAQ LEKELQALEK ENAQLEWELQ
251 ALEKELAQGA T           (SEQ ID NO: 213)

  1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGKGGSAQ LKKKLQALKK KNAQLKWKLQ
251 ALKKKLAQGA T           (SEQ ID NO: 214)
```

In certain aspects, the disclosure relates to type I receptor polypeptides (e.g., type I-Fc fusion proteins) comprising one or more amino acid modifications that alter the isoelectric point (pI) of the type I receptor polypeptide and/or type II receptor polypeptides (e.g., type II-Fc fusion proteins) comprising one or more amino acid modifications that alter the isoelectric point of the type II receptor polypeptide. In some embodiments, one or more candidate domains that have a pI value higher than about 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, or 9.0 are selected for construction of the full multidomain protein. In other embodiments, one or more candidate domains that have a pI value less than about 9.0, 8.5, 8.0, 7.5, 7.0, 6.5, 6.0, 5.5, or 5.0 are selected for construction of the full multidomain protein. It will be understood by one skilled in the art that a single protein will have multiple charge forms. Without wishing to be bound by any particular theory, the charge of a protein can be modified by a number of different mechanisms including but not limited to, amino acid substitution, cationization, deamination, carboxyl-terminal amino acid heterogeneity, phosphorylation and glycosylation.

The pI of a protein may be determined by a variety of methods including but not limited to, isoelectric focusing and various computer algorithms (see for example Bjellqvist et al., 1993, Electrophoresis 14:1023). In one embodiment, pI is determined using a Pharmacia Biotech Multiphor 2 electrophoresis system with a multi temp refrigerated bath recirculation unit and an EPS 3501 XL power supply. Pre-cast ampholine gels (e.g., Amersham Biosciences, pI range 2.5-10) are loaded with protein samples. Broad range pI marker standards (e.g., Amersham, pI range 3-10, 8 .mu.L) are used to determine relative pI for the proteins. Electrophoresis is performed, for example, at 1500 V, 50 mA for 105 minutes. The gel is fixed using, for example, a Sigma fixing solution (5x) diluted with purified water to 1x Staining is performed, for example, overnight at room temperature using Simply Blue stain (Invitrogen). Destaining is carried out, for example, with a solution that consisted of 25% ethanol, 8% acetic acid and 67% purified water. Isoelectric points are determined using, for example, a Bio-Rad Densitometer relative to calibration curves of the standards. The one or more metrics may further include metrics characterizing stability of the domain under one or more different conditions selected from the group consisting of different pH values, different temperatures, different shear stresses, and different freeze/thaw cycles.

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains by methods described above in combination with additional mutations in the Fc domain that facilitate purification of the desired heteromeric species. An example is complementarity of Fc domains based on knobs-into-holes pairing combined with an engineered disulfide bond, as disclosed above, plus additional substitution of two negatively charged amino acids (aspartic acid or glutamic acid) in one Fc-containing polypeptide chain and two positively charged amino acids (e.g., arginine) in the complementary Fc-containing polypeptide chain (SEQ ID NOs: 660 and 670). These four amino acid substitutions facilitate selective purification of the desired heteromeric fusion protein from a heterogeneous polypeptide mixture based on differences in isoelectric point. The engineered amino acid substitutions in these sequences are double underlined below, and the ALK4 or ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 660 or SEQ ID NO: 670, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will

generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 660 and 670).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTENQ VSLWCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQDSLS LSPGK      (SEQ ID NO: 660)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSREEMTKNQ VSLSCAVKGF
151  YPSDIAVEWE SRGQPENNYK TTPPVLDSRG SFFLVSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK      (SEQ ID NO: 670)
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Another example involves complementarity of Fc domains based on knobs-into-holes pairing combined with an engineered disulfide bond, as disclosed above, plus a histidine-to-arginine substitution at position 213 in one Fc-containing polypeptide chain (SEQ ID NO: 680). This substitution (denoted H435R in the numbering system of Kabat et al.) facilitates separation of desired heteromer from undesirable homodimer based on differences in affinity for protein A. The engineered amino acid substitution is indicated by double underline, and the ALK4 or ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 680 or SEQ ID NO: 205, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair of SEQ ID NO: 680 (below) and SEQ ID NO: 205.

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNRYTQKSLS LSPGK     (SEQ ID NO: 680)
```

A variety of engineered mutations in the Fc domain are presented above with respect to the G1Fc sequence (SEQ ID NO: 3100). Analogous mutations in G2Fc, G3Fc, and G4Fc can be derived from their alignment with G1Fc in Figure 5. Due to unequal hinge lengths, analogous Fc positions based on isotype alignment (Figure 5) possess different amino acid numbers in SEQ ID NOs: 3100, 3200, 3300, 3400, and 3500 as summarized in the following table.

Correspondence between C_H3 Positions for Human Fc Isotypes*			
IgG1	IgG4	IgG2	IgG3
SEQ ID NO: 3100	SEQ ID NO: 3500	SEQ ID NO: 3200	SEQ ID NO: 3300
Numbering begins at THT…	Numbering begins at ESK…	Numbering begins at VEC…	Numbering begins at EPK…
Y127	Y131	Y125	Y134
S132	S136	S130	S139
E134	E138	E132	E141
K138	K142	K136	K145
T144	T148	T142	T151
L146	L150	L144	L153
N162	N166	N160	S169
K170	K174	K168	N177
D177	D181	D175	D184
D179	D183	D177	D186
Y185	Y189	Y183	Y192
K187	R191	K185	K194
H213	H217	H211	R220
K217	K221	K215	K224
* Numbering based on multiple sequence alignment shown in Figure 5			

It is understood that different elements of the fusion proteins (*e.g.*, immunoglobulin Fc fusion proteins) may be arranged in any manner that is consistent with desired functionality. For example, a TGF-beta superfamily type I and/or type II receptor polypeptide domain may be placed C-terminal to a heterologous domain, or alternatively, a heterologous domain may be placed C-terminal to a TGF-beta superfamily type I and/or type II receptor polypeptide domain. The TGF-beta superfamily type I and/or type II receptor polypeptide domain and the heterologous domain need not be adjacent in a fusion protein, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains.

For example, a TGF-beta superfamily type I and/or type II receptor fusion protein may comprise an amino acid sequence as set forth in the formula A-B-C. The B portion corresponds to a TGF-beta superfamily type I and/or type II receptor polypeptide domain. The A and C portions may be independently zero, one, or more than one amino acid, and both the A and C portions when present are heterologous to B. The A and/or C portions may be attached to the B portion via a linker sequence. A linker may be rich in glycine (*e.g.*, 2-10, 2-5, 2-4, 2-3 glycine residues) or glycine and proline residues and may, for example, contain a single sequence of threonine/serine and glycines or repeating sequences of threonine/serine and/or glycines, *e.g.*, GGG (SEQ ID NO: 58), GGGG (SEQ ID NO: 59), TGGGG (SEQ ID NO: 60), SGGGG (SEQ ID NO: 61), TGGG (SEQ ID NO: 62), or SGGG (SEQ ID NO: 63) singlets, or repeats. In certain embodiments, a TGF-beta superfamily type I and/or type II receptor fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a leader (signal) sequence, B consists of a TGF-beta superfamily type I and/or type II receptor polypeptide domain, and C is a polypeptide portion that enhances one or more of *in vivo* stability, *in vivo* half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. In certain embodiments, a TGF-beta superfamily type I and/or type II receptor fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a TPA leader sequence, B consists of a TGF-beta superfamily type I and/or type II receptor polypeptide domain, and C is an immunoglobulin Fc domain. Preferred fusion proteins comprise the amino acid sequence set forth in any one of SEQ ID NOs: 100, 102, 104, 106, 112, 114, 115, 117, 118, 120, 121, 123, 124, 126, 127, 129, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, and 416.

In some embodiments, TGF-beta superfamily receptor heteromultimers of the present disclosure further comprise one or more heterologous portions (domains) so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. Well-known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S-transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy-chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt- conjugated resins are used. Many of such matrices are available in "kit" form, such as the Pharmacia GST purification system and the QIAexpress™ system (Qiagen)

useful with (HIS_6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ligand trap polypeptides. Examples of such detection domains include the various fluorescent proteins (*e.g.*, GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well-known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus haemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for factor Xa or thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation.

In certain embodiments, TGF-beta superfamily type I and/or type II receptor polypeptides of the present disclosure comprise one or more modifications that are capable of stabilizing the polypeptides. For example, such modifications enhance the *in vitro* half-life of the polypeptides, enhance circulatory half-life of the polypeptides, and/or reduce proteolytic degradation of the polypeptides. Such stabilizing modifications include, but are not limited to, fusion proteins (including, for example, fusion proteins comprising a TGF-beta superfamily type I and/or type II receptor polypeptide domain and a stabilizer domain), modifications of a glycosylation site (including, for example, addition of a glycosylation site to a polypeptide of the disclosure), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from a polypeptide of the disclosure). As used herein, the term "stabilizer domain" not only refers to a fusion domain (*e.g.*, an immunoglobulin Fc domain) as in the case of fusion proteins, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous moiety, such as polyethylene glycol.

In preferred embodiments, TGF-beta superfamily heteromultimers to be used in accordance with the methods described herein are isolated polypeptide complexes. As used herein, an isolated protein (or protein complex) or polypeptide (or polypeptide complex) is one which has been separated from a component of its natural environment. In some embodiments, a heteromultimer complex of the disclosure is purified to greater than 95%, 96%, 97%, 98%, or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). Methods for assessment of antibody purity are well known in the art [See, *e.g.*, Flatman *et al.*, (2007) J. Chromatogr. B 848:79-87]. In some

embodiments, heteromultimer preparations of the disclosure are substantially free of TGF-beta superfamily type I receptor polypeptide homomultimers and/or TGF-beta superfamily type II receptor polypeptide homomultimers. For example, in some embodiments, heteromultimer preparations comprise less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% of TGF-beta superfamily type I receptor polypeptide homomultimers. In some embodiments, heteromultimer preparations comprise less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% of TGF-beta superfamily type II receptor polypeptide homomultimers. In some embodiments, heteromultimer preparations comprise less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% of TGF-beta superfamily type I receptor polypeptide homomultimers and less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% of TGF-beta superfamily type II receptor polypeptide homomultimers.

In certain embodiments, TGFβ superfamily type I and/or type II receptor polypeptides, as well as heteromultimers thereof, of the disclosure can be produced by a variety of art-known techniques. For example, polypeptides of the disclosure can be synthesized using standard protein chemistry techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant G. A. (ed.), Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). In addition, automated peptide synthesizers are commercially available (see, *e.g.*, Advanced ChemTech Model 396; Milligen/Biosearch 9600). Alternatively, the polypeptides and complexes of the disclosure, including fragments or variants thereof, may be recombinantly produced using various expression systems [*e.g.*, E. coli, Chinese Hamster Ovary (CHO) cells, COS cells, baculovirus] as is well known in the art. In a further embodiment, the modified or unmodified polypeptides of the disclosure may be produced by digestion of recombinantly produced full-length TGFβ superfamily type I and/or type II receptor polypeptides by using, for example, a protease, *e.g.*, trypsin, thermolysin, chymotrypsin, pepsin, or paired basic amino acid converting enzyme (PACE). Computer analysis (using a commercially available software, *e.g.*, MacVector, Omega, PCGene, Molecular Simulation, Inc.) can be used to identify proteolytic cleavage sites.

With respect to antibodies that bind to and antagonize ligands that bind to TGF-beta type I receptor polypeptide:TGF-beta type II receptor polypeptide heteromultimers of the disclosure (e.g., BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C,

activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty) it is contemplated that an antibody may be designed as a bispecific antibody comprising a first portion that binds to an epitope of such ligand, such that the first portion of the antibody competes for binding with a type I receptor and comprising a second portion that binds to an epitope of such ligand, such that the second portion of the antibody competes for binding with a type II receptor. In this manner, a bispecific antibody targeting a single ligand can be designed to mimic the dual type I-type II receptor binding blockade that may be conferred by an ALK7:ActRIIB heteromultimer. Similarly it is contemplated that the same effect could be achieved using a combination of two or more antibodies wherein at least a first antibody binds to an epitope of such ligand, such that the first antibody competes for binding with a type I receptor and at least a second antibody binds to an epitope of such ligand, such that the second antibody competes for binding with a type II receptor.

3. Nucleic Acids Encoding TGFβ superfamily type I and/or type II receptor polypeptides

In certain embodiments, the present disclosure provides isolated and/or recombinant nucleic acids encoding TGFβ superfamily type I and/or type II receptors (including fragments, functional variants, and fusion proteins thereof) disclosed herein. For example, SEQ ID NO: 12 encodes the naturally occurring human ActRIIA precursor polypeptide, while SEQ ID NO: 13 encodes the mature, extracellular domain of ActRIIA. The subject nucleic acids may be single-stranded or double stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making TGF-beta superfamily heteromultimer complexes of the present disclosure.

As used herein, isolated nucleic acid(s) refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

In certain embodiments, nucleic acids encoding TGFβ superfamily type I and/or type II receptor polypeptides of the present disclosure are understood to include nucleic acids that are variants of any one of SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36,

37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions, or deletions including allelic variants, and therefore, will include coding sequences that differ from the nucleotide sequence designated in any one of SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143.

In certain embodiments, TGFβ superfamily type I and/or type II receptor polypeptides of the present disclosure are encoded by isolated or recombinant nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143. One of ordinary skill in the art will appreciate that nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequences complementary to SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143are also within the scope of the present disclosure. In further embodiments, the nucleic acid sequences of the disclosure can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence or in a DNA library.

In other embodiments, nucleic acids of the present disclosure also include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequence designated in SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143, the complement sequence of SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143, or fragments thereof. One of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45 °C, followed by a wash of 2.0 x SSC at 50 °C. For example, the salt concentration in the wash

step can be selected from a low stringency of about 2.0 x SSC at 50 °C to a high stringency of about 0.2 x SSC at 50 °C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22 °C, to high stringency conditions at about 65 °C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In one embodiment, the disclosure provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a wash at 2 x SSC at room temperature.

Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143 due to degeneracy in the genetic code are also within the scope of the disclosure. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this disclosure.

In certain embodiments, the recombinant nucleic acids of the present disclosure may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the disclosure. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a

chromosome. In some embodiments, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

In certain aspects of the present disclosure, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a TGFβ superfamily type I and/or type II receptor polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the TGFβ superfamily type I and/or type II receptor polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; *Gene Expression Technology: Methods in Enzymology*, Academic Press, San Diego, CA (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding a TGFβ superfamily type I and/or type II receptor polypeptides. Such useful expression control sequences, include, for example, the early and late promoters of SV40, *tet* promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the *trp* system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda , the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, *e.g.*, Pho5, the promoters of the yeast α-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

A recombinant nucleic acid of the present disclosure can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression vehicles for production of a recombinant TGFβ superfamily type I and/or type II receptor polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the following types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived

plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see, *e.g.*, Molecular Cloning A Laboratory Manual, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the ß-gal containing pBlueBac III).

In a preferred embodiment, a vector will be designed for production of the subject TGFβ superfamily type I and/or type II receptor polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wisc.). As will be apparent, the subject gene constructs can be used to cause expression of the subject TGFβ superfamily type I and/or type II receptor polypeptide in cells propagated in culture, *e.g.*, to produce proteins, including fusion proteins or variant proteins, for purification.

This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence for one or more of the subject TGFβ superfamily type I and/or type II receptor polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a TGFβ superfamily type I and/or type II receptor polypeptide of the disclosure may

be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells [*e.g.* a Chinese hamster ovary (CHO) cell line]. Other suitable host cells are known to those skilled in the art.

Accordingly, the present disclosure further pertains to methods of producing the subject TGFβ superfamily type I and/or type II receptor polypeptides. For example, a host cell transfected with an expression vector encoding a TGFβ superfamily type I and/or type II receptor polypeptide can be cultured under appropriate conditions to allow expression of the TGFβ superfamily type I and/or type II receptor polypeptide to occur. The polypeptide may be secreted and isolated from a mixture of cells and medium containing the polypeptide. Alternatively, the TGFβ superfamily type I and/or type II receptor polypeptide may be isolated from a cytoplasmic or membrane fraction obtained from harvested and lysed cells. A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art. The subject polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the TGFβ superfamily type I and/or type II receptor polypeptides and affinity purification with an agent that binds to a domain fused to TGFβ superfamily type I and/or type II receptor polypeptide (*e.g.*, a protein A column may be used to purify a TGFβ superfamily type I receptor-Fc and/or type II receptor-Fc fusion protein). In some embodiments, the TGFβ superfamily type I and/or type II receptor polypeptide is a fusion protein containing a domain which facilitates its purification.

In some embodiments, purification is achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. A TGFβ superfamily type I receptor-Fc and/or type II receptor-Fc fusion protein may be purified to a purity of >90%, >95%, >96%, >98%, or >99% as determined by size exclusion chromatography and >90%, >95%, >96%, >98%, or >99% as determined by SDS PAGE. The target level of purity should be one that is sufficient to achieve desirable results in mammalian systems, particularly non-human primates, rodents (mice), and humans.

In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant TGFβ superfamily type I and/or type II receptor polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni^{2+} metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified TGFβ superfamily type I and/or type II receptor polypeptide. *See, e.g.*, Hochuli *et al.* (1987) *J. Chromatography* 411:177; and Janknecht *et al.* (1991) *PNAS USA* 88:8972.

Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence. See, *e.g.*, Current Protocols in Molecular Biology, eds. Ausubel *et al.*, John Wiley & Sons: 1992.

4. Screening Assays

In certain aspects, the present disclosure relates to the use of TGFβ superfamily type I and type II receptor heteromultimers to identify compounds (agents) which are agonists or antagonists of TGFβ superfamily receptors. Compounds identified through this screening can be tested to assess their ability to modulate tissues such as bone, cartilage, muscle, fat, and/or neurons, to assess their ability to modulate tissue growth *in vivo* or *in vitro*. These compounds can be tested, for example, in animal models.

There are numerous approaches to screening for therapeutic agents for modulating tissue growth by targeting TGFβ superfamily ligand signaling (*e.g.*, SMAD 2/3 and/or SMAD 1/5/8 signaling). In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb TGFβ superfamily receptor-mediated effects on a selected cell line. In certain embodiments, the assay is carried out to screen and identify

compounds that specifically inhibit or reduce binding of a TGF-beta superfamily heteromultimer to its binding partner, such as a TGFβ superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). Alternatively, the assay can be used to identify compounds that enhance binding of a TGF-beta superfamily heteromultimer to its binding partner such as a TGFβ superfamily ligand. In a further embodiment, the compounds can be identified by their ability to interact with a TGF-beta superfamily heteromultimer of the disclosure.

A variety of assay formats will suffice and, in light of the present disclosure, those not expressly described herein will nevertheless be comprehended by one of ordinary skill in the art. As described herein, the test compounds (agents) of the invention may be created by any combinatorial chemical method. Alternatively, the subject compounds may be naturally occurring biomolecules synthesized *in vivo* or *in vitro*. Compounds (agents) to be tested for their ability to act as modulators of tissue growth can be produced, for example, by bacteria, yeast, plants or other organisms (*e.g.*, natural products), produced chemically (*e.g.*, small molecules, including peptidomimetics), or produced recombinantly. Test compounds contemplated by the present invention include non-peptidyl organic molecules, peptides, polypeptides, peptidomimetics, sugars, hormones, and nucleic acid molecules. In certain embodiments, the test agent is a small organic molecule having a molecular weight of less than about 2,000 Daltons.

The test compounds of the disclosure can be provided as single, discrete entities, or provided in libraries of greater complexity, such as made by combinatorial chemistry. These libraries can comprise, for example, alcohols, alkyl halides, amines, amides, esters, aldehydes, ethers and other classes of organic compounds. Presentation of test compounds to the test system can be in either an isolated form or as mixtures of compounds, especially in initial screening steps. Optionally, the compounds may be optionally derivatized with other compounds and have derivatizing groups that facilitate isolation of the compounds. Non-limiting examples of derivatizing groups include biotin, fluorescein, digoxygenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S-transferase (GST), photoactivatible crosslinkers or any combinations thereof.

In many drug-screening programs which test libraries of compounds and natural extracts, high-throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity between a TGF-beta superfamily heteromultimer and its binding partner (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty).

Merely to illustrate, in an exemplary screening assay of the present disclosure, the compound of interest is contacted with an isolated and purified TGF-beta superfamily heteromultimer which is ordinarily capable of binding to a TGF-beta superfamily ligand, as appropriate for the intention of the assay. To the mixture of the compound and TGF-beta superfamily heteromultimer is then added to a composition containing the appropriate TGF-beta superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). Detection and quantification of heteromultimer-superfamily ligand provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the TGF-beta superfamily heteromultimer and its binding protein. The efficacy of the compound can be assessed by generating dose-response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. For example, in a control assay, isolated and purified TGF-beta superfamily ligand is added to a composition containing the TGF-beta superfamily heteromultimer, and the formation of heteromultimer-ligand complex is

quantitated in the absence of the test compound. It will be understood that, in general, the order in which the reactants may be admixed can be varied, and can be admixed simultaneously. Moreover, in place of purified proteins, cellular extracts and lysates may be used to render a suitable cell-free assay system.

Binding of a TGF-beta superfamily heteromultimer to another protein may be detected by a variety of techniques. For instance, modulation of the formation of complexes can be quantitated using, for example, detectably labeled proteins such as radiolabeled (*e.g.*, ^{32}P, ^{35}S, ^{14}C or ^{3}H), fluorescently labeled (*e.g.*, FITC), or enzymatically labeled TGF-beta superfamily heteromultimer and/or its binding protein, by immunoassay, or by chromatographic detection.

In certain embodiments, the present disclosure contemplates the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between a TGF-beta superfamily heteromultimer and its binding protein. Further, other modes of detection, such as those based on optical waveguides (see, *e.g.*, PCT Publication WO 96/26432 and U.S. Pat. No. 5,677,196), surface plasmon resonance (SPR), surface charge sensors, and surface force sensors, are compatible with many embodiments of the disclosure.

Moreover, the present disclosure contemplates the use of an interaction trap assay, also known as the "two-hybrid assay," for identifying agents that disrupt or potentiate interaction between a TGF-beta superfamily heteromultimer and its binding partner. See, *e.g.*, U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) Cell 72:223-232; Madura *et al.* (1993) J Biol Chem 268:12046-12054; Bartel *et al.* (1993) Biotechniques 14:920-924; and Iwabuchi *et al.* (1993) Oncogene 8:1693-1696). In a specific embodiment, the present disclosure contemplates the use of reverse two-hybrid systems to identify compounds (*e.g.*, small molecules or peptides) that dissociate interactions between a TGF-beta superfamily heteromultimer and its binding protein [see, *e.g.*, Vidal and Legrain, (1999) Nucleic Acids Res 27:919-29; Vidal and Legrain, (1999) Trends Biotechnol 17:374-81; and U.S. Pat. Nos. 5,525,490; 5,955,280; and 5,965,368].

In certain embodiments, the subject compounds are identified by their ability to interact with a TGF-beta superfamily heteromultimer of the disclosure. The interaction between the compound and the TGF-beta superfamily heteromultimer may be covalent or non-covalent. For example, such interaction can be identified at the protein level using *in*

vitro biochemical methods, including photo-crosslinking, radiolabeled ligand binding, and affinity chromatography. See, *e.g.*, Jakoby WB *et al.* (1974) Methods in Enzymology 46:1. In certain cases, the compounds may be screened in a mechanism-based assay, such as an assay to detect compounds which bind to a TGF-beta superfamily heteromultimer. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding a TGF-beta superfamily heteromultimer can be transfected with a reporter system (*e.g.*, β-galactosidase, luciferase, or green fluorescent protein) into a cell and screened against the library preferably by high-throughput screening or with individual members of the library. Other mechanism-based binding assays may be used; for example, binding assays which detect changes in free energy. Binding assays can be performed with the target fixed to a well, bead or chip or captured by an immobilized antibody or resolved by capillary electrophoresis. The bound compounds may be detected usually using colorimetric endpoints or fluorescence or surface plasmon resonance.

5. Exemplary Therapeutic Uses

In certain embodiments, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the present disclosure can be used to treat or prevent a disease or condition that is associated with abnormal activity of a TGFβ superfamily receptor (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, ActRIIB, BMPRII, TGFBRII, and MISRII) and/or a TGFβ superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). These diseases, disorders or conditions are generally referred to herein as "TGFβ superfamily-associated conditions" or "TGFβ superfamily-associated disorders." In certain embodiments, the present invention provides methods of treating or preventing an individual in need thereof through administering to the individual a therapeutically effective amount of a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, as described herein. Any of the TGF-beta superfamily heteromultimer complexes of the present disclosure can potentially be employed individually or in combination for therapeutic uses disclosed

herein. These methods are particularly aimed at therapeutic and prophylactic treatments of mammals including, for example, rodents, primates, and humans.

As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample. The term "treating" as used herein includes amelioration or elimination of the condition once it has been established. In either case, prevention or treatment may be discerned in the diagnosis provided by a physician or other health care provider and the intended result of administration of the therapeutic agent.

TGFβ superfamily receptor-ligand complexes play essential roles in tissue growth as well as early developmental processes such as the correct formation of various structures or in one or more post-developmental capacities including sexual development, pituitary hormone production, and creation of bone and cartilage. Thus, TGFβ superfamily-associated conditions/disorders include abnormal tissue growth and developmental defects. In addition, TGFβ superfamily-associated conditions include, but are not limited to, disorders of cell growth and differentiation such as inflammation, allergy, autoimmune diseases, infectious diseases, and tumors.

Exemplary TGFβ superfamily-associated conditions include neuromuscular disorders (*e.g.*, muscular dystrophy and muscle atrophy), congestive obstructive pulmonary disease (and muscle wasting associated with COPD), muscle wasting syndrome, sarcopenia, cachexia, adipose tissue disorders (*e.g.*, obesity), type 2 diabetes (NIDDM, adult-onset diabetes), and bone degenerative disease (*e.g.*, osteoporosis). Other exemplary TGFβ superfamily-associated conditions include musculodegenerative and neuromuscular disorders, tissue repair (*e.g.*, wound healing), neurodegenerative diseases (*e.g.*, amyotrophic lateral sclerosis), and immunologic disorders (*e.g.*, disorders related to abnormal proliferation or function of lymphocytes).

In certain embodiments, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the disclosure are used as part of a treatment for a muscular dystrophy. The term "muscular dystrophy" refers to a group of degenerative muscle diseases characterized by gradual weakening and deterioration of skeletal muscles and sometimes the heart and respiratory muscles. Muscular dystrophies are genetic disorders

characterized by progressive muscle wasting and weakness that begin with microscopic changes in the muscle. As muscles degenerate over time, the person's muscle strength declines. Exemplary muscular dystrophies that can be treated with a regimen including the subject TGF-beta superfamily heteromultimer complexes include: Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), Emery-Dreifuss muscular dystrophy (EDMD), limb-girdle muscular dystrophy (LGMD), facioscapulohumeral muscular dystrophy (FSH or FSHD) (also known as Landouzy-Dejerine), myotonic dystrophy (MMD; also known as Steinert's Disease), oculopharyngeal muscular dystrophy (OPMD), distal muscular dystrophy (DD), congenital muscular dystrophy (CMD).

Duchenne muscular dystrophy (DMD) was first described by the French neurologist Guillaume Benjamin Amand Duchenne in the 1860s. Becker muscular dystrophy (BMD) is named after the German doctor Peter Emil Becker, who first described this variant of DMD in the 1950s. DMD is one of the most frequent inherited diseases in males, affecting one in 3,500 boys. DMD occurs when the dystrophin gene, located on the short arm of the X chromosome, is defective. Since males only carry one copy of the X chromosome, they only have one copy of the dystrophin gene. Without the dystrophin protein, muscle is easily damaged during cycles of contraction and relaxation. While early in the disease muscle compensates by regeneration, later on muscle progenitor cells cannot keep up with the ongoing damage and healthy muscle is replaced by non-functional fibro-fatty tissue.

BMD results from different mutations in the dystrophin gene. BMD patients have some dystrophin, but it is either of insufficient quantity or poor quality. The presence of some dystrophin protects the muscles of patients with BMD from degenerating as severely or as quickly as those of patients with DMD.

Studies in animals indicate that inhibition of the GDF8 signaling pathway may effectively treat various aspects of disease in DMD and BMD patients (Bogdanovich et al., 2002, Nature 420:418-421; Pistilli et al., 2011, Am J Pathol 178:1287-1297). Thus, TGF-beta superfamily heteromultimer complexes of the disclosure may act as GDF8 inhibitors (antagonists), and constitute an alternative means of blocking signaling by GDF8 and/or related TGFβ superfamily ligands *in vivo* in DMD and BMD patients.

Similarly, TGF-beta superfamily heteromultimers of the disclosure may provide an effective means to increase muscle mass in other disease conditions that are in need of muscle growth. For example, amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's

disease or motor neuron disease, is a chronic, progressive, and incurable CNS disorder that attacks motor neurons, which are components of the central nervous system required for initiation of skeletal muscle contraction. In ALS, motor neurons deteriorate and eventually die, and though a person's brain normally remains fully functioning and alert, initiation of muscle contraction is blocked at the spinal level. Individuals who develop ALS are typically between 40 and 70 years old, and the first motor neurons to degenerate are those innervating the arms or legs. Patients with ALS may have trouble walking, may drop things, fall, slur their speech, and laugh or cry uncontrollably. As the disease progresses, muscles in the limbs begin to atrophy from disuse. Muscle weakness becomes debilitating, and patients eventually require a wheel chair or become confined to bed. Most ALS patients die from respiratory failure or from complications of ventilator assistance like pneumonia 3-5 years from disease onset.

Promotion of increased muscle mass by TGF-beta superfamily heteromultimers might also benefit those suffering from muscle wasting diseases. Gonzalez-Cadavid *et al.* (supra) reported that GDF8 expression correlates inversely with fat-free mass in humans and that increased expression of the GDF8 gene is associated with weight loss in men with AIDS wasting syndrome. By inhibiting the function of GDF8 in AIDS patients, at least certain symptoms of AIDS may be alleviated, if not completely eliminated, thus significantly improving quality of life in AIDS patients.

Since loss of GDF8 function is also associated with fat loss without diminution of nutrient intake (Zimmers et al., supra; McPherron and Lee, supra), the subject TGF-beta superfamily heteromultimers may further be used as a therapeutic agent for slowing or preventing the development of obesity and type 2 diabetes.

Cancer anorexia-cachexia syndrome is among the most debilitating and life-threatening aspects of cancer. This syndrome is a common feature of many types of cancer – present in approximately 80% of cancer patients at death – and is responsible not only for a poor quality of life and poor response to chemotherapy but also a shorter survival time than is found in patients with comparable tumors but without weight loss. Cachexia is typically suspected in patients with cancer if an involuntary weight loss of greater than five percent of premorbid weight occurs within a six-month period. Associated with anorexia, wasting of fat and muscle tissue, and psychological distress, cachexia arises from a complex interaction between the cancer and the host. Cancer cachexia affects cytokine production, release of lipid-mobilizing and proteolysis-inducing factors, and alterations in intermediary metabolism.

Although anorexia is common, a decreased food intake alone is unable to account for the changes in body composition seen in cancer patients, and increasing nutrient intake is unable to reverse the wasting syndrome. Currently, there is no treatment to control or reverse the cachexic process. Since systemic overexpression of GDF8 in adult mice was found to induce profound muscle and fat loss analogous to that seen in human cachexia syndromes (Zimmers et al., supra), the subject TGF-beta superfamily heteromultimer complex pharmaceutical compositions may be beneficially used to prevent, treat, or alleviate the symptoms of the cachexia syndrome, where muscle growth is desired. An example of a heteromultimer useful for preventing, treating, or alleviating muscle loss as described above is ActRIIB-Fc:ALK4-Fc.

In certain embodiments, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the present disclosure may be used in methods of inducing bone and/or cartilage formation, preventing bone loss, increasing bone mineralization, preventing the demineralization of bone, and/or increasing bone density. TGF-beta superfamily heteromultimer complexes may be useful in patients who are diagnosed with subclinical low bone density, as a protective measure against the development of osteoporosis.

In some embodiments, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the present disclosure may find medical utility in the healing of bone fractures and cartilage defects in humans and other animals. The subject methods and compositions may also have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent is useful for repair of craniofacial defects that are congenital, trauma-induced, or caused by oncologic resection, and is also useful in cosmetic plastic surgery. Further, methods and compositions of the invention may be used in the treatment of periodontal disease and in other tooth repair processes. In certain cases, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells, or induce differentiation of progenitors of bone-forming cells. TGF-beta superfamily heteromultimers of the disclosure may also be useful in the treatment of osteoporosis. Further, TGF-beta superfamily heteromultimers may be used in repair of cartilage defects and prevention/reversal of osteoarthritis. Examples of heteromultimers useful for inducing bone formation, preventing bone loss, increasing bone mineralization, preventing the

demineralization of bone, and/or increasing bone density as described above are ActRIIB-Fc:ALK3-Fc and ActRIIB-Fc:ALK4-Fc.

Methods and compositions of the invention can be applied to conditions characterized by or causing bone loss, such as osteoporosis (including secondary osteoporosis), hyperparathyroidism, chronic kidney disease mineral bone disorder, sex hormone deprivation or ablation (e.g. androgen and/or estrogen), glucocorticoid treatment, rheumatoid arthritis, severe burns, hyperparathyroidism, hypercalcemia, hypocalcemia, hypophosphatemia, osteomalacia (including tumor-induced osteomalacia), hyperphosphatemia, vitamin D deficiency, hyperparathyroidism (including familial hyperparathyroidism) and pseudohypoparathyroidism, tumor metastases to bone, bone loss as a consequence of a tumor or chemotherapy, tumors of the bone and bone marrow (e.g., multiple myeloma), ischemic bone disorders, periodontal disease and oral bone loss, Cushing's disease, Paget's disease, thyrotoxicosis, chronic diarrheal state or malabsorption, renal tubular acidosis, or anorexia nervosa. Methods and compositions of the invention may also be applied to conditions characterized by a failure of bone formation or healing, including non-union fractures, fractures that are otherwise slow to heal, fetal and neonatal bone dysplasias (e.g., hypocalcemia, hypercalcemia, calcium receptor defects and vitamin D deficiency), osteonecrosis (including osteonecrosis of the jaw) and osteogenesis imperfecta. Additionally, the anabolic effects will cause such antagonists to diminish bone pain associated with bone damage or erosion. As a consequence of the anti-resorptive effects, such antagonists may be useful to treat disorders of abnormal bone formation, such as osteoblastic tumor metastases (e.g., associated with primary prostate or breast cancer), osteogenic osteosarcoma, osteopetrosis, progressive diaphyseal dysplasia, endosteal hyperostosis, osteopoikilosis, and melorheostosis. Other disorders that may be treated include fibrous dysplasia and chondrodysplasias.

In another specific embodiment, the disclosure provides a therapeutic method and composition for repairing fractures and other conditions related to cartilage and/or bone defects or periodontal diseases. The invention further provides therapeutic methods and compositions for wound healing and tissue repair. The types of wounds include, but are not limited to, burns, incisions and ulcers. See, *e.g.*, PCT Publication No. WO 84/01106. Such compositions comprise a therapeutically effective amount of at least one of the TGF-beta superfamily heteromultimer complexes of the disclosure in admixture with a pharmaceutically acceptable vehicle, carrier, or matrix.

In some embodiments, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the disclosure can be applied to conditions causing bone loss such as osteoporosis, hyperparathyroidism, Cushing's disease, thyrotoxicosis, chronic diarrheal state or malabsorption, renal tubular acidosis, or anorexia nervosa. It is commonly appreciated that being female, having a low body weight, and leading a sedentary lifestyle are risk factors for osteoporosis (loss of bone mineral density, leading to fracture risk). However, osteoporosis can also result from the long-term use of certain medications. Osteoporosis resulting from drugs or another medical condition is known as secondary osteoporosis. In Cushing's disease, the excess amount of cortisol produceds by the body results in osteoporosis and fractures. The most common medications associated with secondary osteoporosis are the corticosteroids, a class of drugs that act like cortisol, a hormone produced naturally by the adrenal glands. Although adequate levels of thyroid hormones are needed for the development of the skeleton, excess thyroid hormone can decrease bone mass over time. Antacids that contain aluminum can lead to bone loss when taken in high doses by people with kidney problems, particularly those undergoing dialysis. Other medications that can cause secondary osteoporosis include phenytoin (Dilantin) and barbiturates that are used to prevent seizures; methotrexate (Rheumatrex, Immunex, Folex PFS), a drug for some forms of arthritis, cancer, and immune disorders; cyclosporine (Sandimmune, Neoral), a drug used to treat some autoimmune diseases and to suppress the immune system in organ transplant patients; luteinizing hormone-releasing hormone agonists (Lupron, Zoladex), used to treat prostate cancer and endometriosis; heparin (Calciparine, Liquaemin), an anticlotting medication; and cholestyramine (Questran) and colestipol (Colestid), used to treat high cholesterol. Bone loss resulting from cancer therapy is widely recognized and termed cancer therapy-induced bone loss (CTIBL). Bone metastases can create cavities in the bone that may be corrected by treatment with a TGF-beta superfamily heteromultimer. Bone loss can also be caused by gum disease, a chronic infection in which bacteria located in gum recesses produce toxins and harmful enzymes.

In a further embodiment, the present disclosure provides methods and therapeutic agents for treating diseases or disorders associated with abnormal or unwanted bone growth. For example, patients with the congenital disorder fibrodysplasia ossificans progressiva (FOP) are afflicted by progressive ectopic bone growth in soft tissues spontaneously or in response to tissue trauma, with a major impact on quality of life. Additionally, abnormal bone growth can occur after hip replacement surgery and thus ruin the surgical outcome. This is a more

common example of pathological bone growth and a situation in which the subject methods and compositions may be therapeutically useful. The same methods and compositions may also be useful for treating other forms of abnormal bone growth (e.g., pathological growth of bone following trauma, burns or spinal cord injury), and for treating or preventing the undesirable conditions associated with the abnormal bone growth seen in connection with metastatic prostate cancer or osteosarcoma.

In certain embodiments, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the disclosure may be used to promote bone formation in patients with cancer. Patients having certain tumors (e.g. prostate, breast, multiple myeloma or any tumor causing hyperparathyroidism) are at high risk for bone loss due to tumor-induced bone loss, bone metastases, and therapeutic agents. Such patients may be treated with a TGF-beta superfamily heteromultimer, or a combination of heteromultimers, even in the absence of evidence of bone loss or bone metastases. Patients may also be monitored for evidence of bone loss or bone metastases, and may be treated with a TGF-beta superfamily heteromultimer in the event that indicators suggest an increased risk. Generally, DEXA scans are employed to assess changes in bone density, while indicators of bone remodeling may be used to assess the likelihood of bone metastases. Serum markers may be monitored. Bone specific alkaline phosphatase (BSAP) is an enzyme that is present in osteoblasts. Blood levels of BSAP are increased in patients with bone metastasis and other conditions that result in increased bone remodeling. Osteocalcin and procollagen peptides are also associated with bone formation and bone metastases. Increases in BSAP have been detected in patients with bone metastasis caused by prostate cancer, and to a lesser degree, in bone metastases from breast cancer. BMP7 levels are high in prostate cancer that has metastasized to bone, but not in bone metastases due to bladder, skin, liver, or lung cancer. Type I carboxy-terminal telopeptide (ICTP) is a crosslink found in collagen that is formed during to the resorption of bone. Since bone is constantly being broken down and reformed, ICTP will be found throughout the body. However, at the site of bone metastasis, the level will be significantly higher than in an area of normal bone. ICTP has been found in high levels in bone metastasis due to prostate, lung, and breast cancer. Another collagen crosslink, Type I N-terminal telopeptide (NTx), is produced along with ICTP during bone turnover. The amount of NTx is increased in bone metastasis caused by many different types of cancer including lung, prostate, and breast cancer. Also, the levels of NTx increase with the progression of the bone metastasis. Therefore, this marker can be used to both detect

metastasis as well as measure the extent of the disease. Other markers of resorption include pyridinoline and deoxypyridinoline. Any increase in resorption markers or markers of bone metastases indicate the need for therapy with a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily heteromultimer complexes, in a patient.

A TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the disclosure may be conjointly administered with other bone-active pharmaceutical agents. Conjoint administration may be accomplished by administration of a single co-formulation, by simultaneous administration, or by administration at separate times. TGF-beta superfamily heteromultimers may be particularly advantageous if administered with other bone-active agents. A patient may benefit from conjointly receiving a TGF-beta superfamily heteromultimer and taking calcium supplements, vitamin D, appropriate exercise and/or, in some cases, other medication. Examples of other medications incude, bisphosphonates (alendronate, ibandronate and risedronate), calcitonin, estrogens, parathyroid hormone and raloxifene. The bisphosphonates (alendronate, ibandronate and risedronate), calcitonin, estrogens and raloxifene affect the bone remodeling cycle and are classified as anti-resorptive medications. Bone remodeling consists of two distinct stages: bone resorption and bone formation. Anti-resorptive medications slow or stop the bone-resorbing portion of the bone-remodeling cycle but do not slow the bone-forming portion of the cycle. As a result, new formation continues at a greater rate than bone resorption, and bone density may increase over time. Teriparatide, a form of parathyroid hormone, increases the rate of bone formation in the bone remodeling cycle. Alendronate is approved for both the prevention (5 mg per day or 35 mg once a week) and treatment (10 mg per day or 70 mg once a week) of postmenopausal osteoporosis. Alendronate reduces bone loss, increases bone density and reduces the risk of spine, wrist and hip fractures. Alendronate also is approved for treatment of glucocorticoid-induced osteoporosis in men and women as a result of long-term use of these medications (i.e., prednisone and cortisone) and for the treatment of osteoporosis in men. Alendronate plus vitamin D is approved for the treatment of osteoporosis in postmenopausal women (70 mg once a week plus vitamin D), and for treatment to improve bone mass in men with osteoporosis. Ibandronate is approved for the prevention and treatment of postmenopausal osteoporosis. Taken as a once-a-month pill (150 mg), ibandronate should be taken on the same day each month. Ibandronate reduces bone loss, increases bone density and reduces the risk of spine fractures. Risedronate is approved for the prevention and treatment of postmenopausal osteoporosis. Taken daily (5 mg dose) or

weekly (35 mg dose or 35 mg dose with calcium), risedronate slows bone loss, increases bone density and reduces the risk of spine and non-spine fractures. Risedronate also is approved for use by men and women to prevent and/or treat glucocorticoid-induced osteoporosis that results from long-term use of these medications (i.e., prednisone or cortisone). Calcitonin is a naturally occurring hormone involved in calcium regulation and bone metabolism. In women who are more than 5 years beyond menopause, calcitonin slows bone loss, increases spinal bone density, and may relieve the pain associated with bone fractures. Calcitonin reduces the risk of spinal fractures. Calcitonin is available as an injection (50-100 IU daily) or nasal spray (200 IU daily).

A patient may also benefit from conjointly receiving a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, and additional bone-active medications. Estrogen therapy (ET)/hormone therapy (HT) is approved for the prevention of osteoporosis. ET has been shown to reduce bone loss, increase bone density in both the spine and hip, and reduce the risk of hip and spinal fractures in postmenopausal women. ET is administered most commonly in the form of a pill or skin patch that delivers a low dose of approximately 0.3 mg daily or a standard dose of approximately 0.625 mg daily and is effective even when started after age 70. When estrogen is taken alone, it can increase a woman's risk of developing cancer of the uterine lining (endometrial cancer). To eliminate this risk, healthcare providers prescribe the hormone progestin in combination with estrogen (hormone replacement therapy or HT) for those women who have an intact uterus. ET/HT relieves menopause symptoms and has been shown to have a beneficial effect on bone health. Side effects may include vaginal bleeding, breast tenderness, mood disturbances and gallbladder disease. Raloxifene, 60 mg a day, is approved for the prevention and treatment of postmenopausal osteoporosis. It is from a class of drugs called Selective Estrogen Receptor Modulators (SERMs) that have been developed to provide the beneficial effects of estrogens without their potential disadvantages. Raloxifene increases bone mass and reduces the risk of spine fractures. Data are not yet available to demonstrate that raloxifene can reduce the risk of hip and other non-spine fractures. Teriparatide, a form of parathyroid hormone, is approved for the treatment of osteoporosis in postmenopausal women and men who are at high risk for a fracture. This medication stimulates new bone formation and significantly increases bone mineral density. In postmenopausal women, fracture reduction was noted in the spine, hip, foot, ribs and wrist. In men, fracture reduction was noted in the spine, but

there were insufficient data to evaluate fracture reduction at other sites. Teriparatide is self-administered as a daily injection for up to 24 months.

In other embodiments, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers can be used for regulating body fat content in an animal and for treating or preventing conditions related thereto, and particularly, health-compromising conditions related thereto. According to the present invention, to regulate (control) body weight can refer to reducing or increasing body weight, reducing or increasing the rate of weight gain, or increasing or reducing the rate of weight loss, and also includes actively maintaining, or not significantly changing body weight (*e.g.*, against external or internal influences which may otherwise increase or decrease body weight). One embodiment of the present disclosure relates to regulating body weight by administering to an animal (*e.g.*, a human) in need thereof a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the disclosure.

In some embodiments, a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the present disclosure can be used for reducing body weight and/or reducing weight gain in an animal, and more particularly, for treating or ameliorating obesity in patients at risk for or suffering from obesity. In another specific embodiment, the present invention is directed to methods and compounds for treating an animal that is unable to gain or retain weight (*e.g.*, an animal with a wasting syndrome). Such methods are effective to increase body weight and/or mass, or to reduce weight and/or mass loss, or to improve conditions associated with or caused by undesirably low (*e.g.*, unhealthy) body weight and/or mass. In addition, disorders of high cholesterol (e.g., hypercholesterolemia or dislipidemia) may be treated with a TGF-beta superfamily heteromultimer, or combinations of TGF-beta superfamily heteromultimers, of the disclosure.

In certain aspects, a TGF-beta superfamily heteromultimer, or a combination of TGF-beta superfamily heteromultimers, of the present disclosure can be used to increase red blood cell levels, treat or prevent an anemia, and/or treat or prevent ineffective erythropoiesis in a subject in need thereof. In certain aspects, a TGF-beta superfamily heteromultimer, or a combination of TGF-beta superfamily heteromultimers, of the present disclosure may be used in combination with conventional therapeutic approaches for increasing red blood cell levels, particularly those used to treat anemias of multifactorial origin. Conventional therapeutic approaches for increasing red blood cell levels include, for example, red blood cell transfusion, administration of one or more EPO receptor activators, hematopoietic stem cell

transplantation, immunosuppressive biologics and drugs (*e.g.*, corticosteroids). In certain embodiments, the patient has an anemia and is non-responsive or intolerate to treatment with EPO (or a derivative thereof or an EPO receptor agonist) In certain embodiments, a TGF-beta superfamily heteromultimer, or a combination of TGF-beta superfamily heteromultimers, of the present disclosure can be used to treat or prevent ineffective erythropoiesis and/or the disorders associated with ineffective erythropoiesis in a subject in need thereof. In certain aspects, a TGF-beta superfamily heteromultimer, or a combination of TGF-beta superfamily heteromultimers, of the present disclosure can be used in combination with conventional therapeutic approaches for treating or preventing an anemia or ineffective erythropoiesis disorder, particularly those used to treat anemias of multifactorial origin.

In general, treatment or prevention of a disease or condition as described in the present disclosure is achieved by administering a TGF-beta superfamily heteromultimer, or a combination of TGF-beta superfamily heteromultimers, of the present disclosure in an "effective amount". An effective amount of an agent refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of an agent of the present disclosure may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the agent to elicit a desired response in the individual. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result.

In certain embodiments, a TGF-beta superfamily heteromultimer, or a combination of TGF-beta superfamily heteromultimers, optionally combined with an EPO receptor activator, may be used to increase red blood cell, hemoglobin, or reticulocyte levels in healthy individuals and selected patient populations. Examples of appropriate patient populations include those with undesirably low red blood cell or hemoglobin levels, such as patients having an anemia, and those that are at risk for developing undesirably low red blood cell or hemoglobin levels, such as those patients who are about to undergo major surgery or other procedures that may result in substantial blood loss. In one embodiment, a patient with adequate red blood cell levels is treated with a TGF-beta superfamily heteromultimer, or a combination of TGF-beta superfamily heteromultimers, to increase red blood cell levels, and then blood is drawn and stored for later use in transfusions.

One or more TGF-beta superfamily heteromultimers of the disclosure, optionally combined with an EPO receptor activator, may be used to increase red blood cell levels,

hemoglobin levels, and/or hematocrit levels in a patient having an anemia. When observing hemoglobin and/or hematocrit levels in humans, a level of less than normal for the appropriate age and gender category may be indicative of anemia, although individual variations are taken into account. For example, a hemoglobin level from 10-12.5 g/dl, and typically about 11.0 g/dl is considered to be within the normal range in health adults, although, in terms of therapy, a lower target level may cause fewer cardiovascular side effects [see, *e.g.*, Jacobs et al. (2000) Nephrol Dial Transplant 15, 15-19]. Alternatively, hematocrit levels (percentage of the volume of a blood sample occupied by the cells) can be used as a measure for anemia. Hematocrit levels for healthy individuals range from about 41-51% for adult males and from 35-45% for adult females. In certain embodiments, a patient may be treated with a dosing regimen intended to restore the patient to a target level of red blood cells, hemoglobin, and/or hematocrit. As hemoglobin and hematocrit levels vary from person to person, optimally, the target hemoglobin and/or hematocrit level can be individualized for each patient.

Anemia is frequently observed in patients having a tissue injury, an infection, and/or a chronic disease, particularly cancer. In some subjects, anemia is distinguished by low erythropoietin levels and/or an inadequate response to erythropoietin in the bone marrow [see, *e.g.*, Adamson (2008) Harrison's Principles of Internal Medicine, 17th ed.; McGraw Hill, New York, pp 628-634]. Potential causes of anemia include, for example, blood loss, nutritional deficits (*e.g.* reduced dietary intake of protein), medication reaction, various problems associated with the bone marrow, and many diseases. More particularly, anemia has been associated with a variety of disorders and conditions that include, for example, bone marrow transplantation; solid tumors (*e.g.*, breast cancer, lung cancer, and colon cancer); tumors of the lymphatic system (*e.g.*, chronic lymphocyte leukemia, non-Hodgkins lymphoma, and Hodgkins lymphoma); tumors of the hematopoietic system (*e.g.*, leukemia, a myelodysplastic syndrome and multiple myeloma); radiation therapy; chemotherapy (*e.g.*, platinum containing regimens); inflammatory and autoimmune diseases, including, but not limited to, rheumatoid arthritis, other inflammatory arthritides, systemic lupus erythematosis (SLE), acute or chronic skin diseases (*e.g.*, psoriasis), inflammatory bowel disease (*e.g.*, Crohn's disease and ulcerative colitis); acute or chronic renal disease or failure, including idiopathic or congenital conditions; acute or chronic liver disease; acute or chronic bleeding; situations where transfusion of red blood cells is not possible due to patient allo- or auto-antibodies and/or for religious reasons (*e.g.*, some Jehovah's Witnesses); infections (*e.g.*,

malaria and osteomyelitis); hemoglobinopathies including, for example, sickle cell disease (anemia), thalassemias; drug use or abuse (*e.g.*, alcohol misuse); pediatric patients with anemia from any cause to avoid transfusion; and elderly patients or patients with underlying cardiopulmonary disease with anemia who cannot receive transfusions due to concerns about circulatory overload [see, *e.g.*, Adamson (2008) Harrison's Principles of Internal Medicine, 17th ed.; McGraw Hill, New York, pp 628-634]. In some embodiments, one or more TGF-beta superfamily heteromultimers of the disclosure could be used to treat or prevent anemia associated with one or more of the disorders or conditions disclosed herein.

Many factors can contribute to cancer-related anemia. Some are associated with the disease process itself and the generation of inflammatory cytokines such as interleukin-1, interferon-gamma, and tumor necrosis factor [Bron *et al.* (2001) Semin Oncol 28(Suppl 8):1-6]. Among its effects, inflammation induces the key iron-regulatory peptide hepcidin, thereby inhibiting iron export from macrophages and generally limiting iron availability for erythropoiesis [see, *e.g.*, Ganz (2007) J Am Soc Nephrol 18:394-400]. Blood loss through various routes can also contribute to cancer-related anemia. The prevalence of anemia due to cancer progression varies with cancer type, ranging from 5% in prostate cancer up to 90% in multiple myeloma. Cancer-related anemia has profound consequences for patients, including fatigue and reduced quality of life, reduced treatment efficacy, and increased mortality. In some embodiments, one or more TGF-beta superfamily heteromultimers of the disclosure, optionally combined with an EPO receptor activator, could be used to treat a cancer-related anemia.

A hypoproliferative anemia can result from primary dysfunction or failure of the bone marrow. Hypoproliferative anemias include: anemia of chronic disease, anemia of kidney disease, anemia associated with hypometabolic states, and anemia associated with cancer. In each of these types, endogenous erythropoietin levels are *inappropriately low* for the degree of anemia observed. Other hypoproliferative anemias include: early-stage iron-deficient anemia, and anemia caused by damage to the bone marrow. In these types, endogenous erythropoietin levels are *appropriately elevated* for the degree of anemia observed. Prominent examples would be myelosuppression caused by cancer and/or chemotherapeutic drugs or cancer radiation therapy. A broad review of clinical trials found that mild anemia can occur in 100% of patients after chemotherapy, while more severe anemia can occur in up to 80% of such patients [see, *e.g.*, Groopman *et al.* (1999) J Natl Cancer Inst 91:1616-1634]. Myelosuppressive drugs include, for example: 1) alkylating agents such as nitrogen mustards

(*e.g.*, melphalan) and nitrosoureas (*e.g.*, streptozocin); 2) antimetabolites such as folic acid antagonists (*e.g.*, methotrexate), purine analogs (*e.g.*, thioguanine), and pyrimidine analogs (*e.g.*, gemcitabine); 3) cytotoxic antibiotics such as anthracyclines (*e.g.*, doxorubicin); 4) kinase inhibitors (*e.g.*, gefitinib); 5) mitotic inhibitors such as taxanes (*e.g.*, paclitaxel) and vinca alkaloids (*e.g.*, vinorelbine); 6) monoclonal antibodies (*e.g.*, rituximab); and 7) topoisomerase inhibitors (*e.g.*, topotecan and etoposide). In addition, conditions resulting in a hypometabolic rate can produce a mild-to-moderate hypoproliferative anemia. Among such conditions are endocrine deficiency states. For example, anemia can occur in Addison's disease, hypothyroidism, hyperparathyroidism, or males who are castrated or treated with estrogen. In some embodiments, one or more TGF-beta superfamily heteromultimer complexes of the disclosure, optionally combined with an EPO receptor activator, could be used to treat a hyperproliferative anemia.

Chronic kidney disease is sometimes associated with hypoproliferative anemia, and the degree of the anemia varies in severity with the level of renal impairment. Such anemia is primarily due to inadequate production of erythropoietin and reduced survival of red blood cells. Chronic kidney disease usually proceeds gradually over a period of years or decades to end-stage (Stage 5) disease, at which point dialysis or kidney transplantation is required for patient survival. Anemia often develops early in this process and worsens as disease progresses. The clinical consequences of anemia of kidney disease are well-documented and include development of left ventricular hypertrophy, impaired cognitive function, reduced quality of life, and altered immune function [see, *e.g.*, Levin *et al.* (1999) Am J Kidney Dis 27:347-354; Nissenson (1992) Am J Kidney Dis 20(Suppl 1):21-24; Revicki *et al.* (1995) Am J Kidney Dis 25:548-554; Gafter *et al.*, (1994) Kidney Int 45:224-231]. In some embodiments, one or more TGF-beta superfamily heteromultimer complexes of the disclosure, optionally combined with an EPO receptor activator, could be used to treat anemia associated with acute or chronic renal disease or failure.

Anemia resulting from acute blood loss of sufficient volume, such as from trauma or postpartum hemorrhage, is known as acute post-hemorrhagic anemia. Acute blood loss initially causes hypovolemia without anemia since there is proportional depletion of RBCs along with other blood constituents. However, hypovolemia will rapidly trigger physiologic mechanisms that shift fluid from the extravascular to the vascular compartment, which results in hemodilution and anemia. If chronic, blood loss gradually depletes body iron stores and eventually leads to iron deficiency. In some embodiments, one or more TGF-beta

superfamily heteromultimers of the disclosure, optionally combined with an EPO receptor activator, could be used to treat anemia resulting from acute blood loss.

Iron-deficiency anemia is the final stage in a graded progression of increasing iron deficiency which includes negative iron balance and iron-deficient erythropoiesis as intermediate stages. Iron deficiency can result from increased iron demand, decreased iron intake, or increased iron loss, as exemplified in conditions such as pregnancy, inadequate diet, intestinal malabsorption, acute or chronic inflammation, and acute or chronic blood loss. With mild-to-moderate anemia of this type, the bone marrow remains hypoproliferative, and RBC morphology is largely normal; however, even mild anemia can result in some microcytic hypochromic RBCs, and the transition to severe iron-deficient anemia is accompanied by hyperproliferation of the bone marrow and increasingly prevalent microcytic and hypochromic RBCs [see, *e.g.*, Adamson (2008) Harrison's Principles of Internal Medicine, 17th ed.; McGraw Hill, New York, pp 628-634]. Appropriate therapy for iron-deficiency anemia depends on its cause and severity, with oral iron preparations, parenteral iron formulations, and RBC transfusion as major conventional options. In some embodiments, one or more TGF-beta superfamily heteromultimers of the disclosure, optionally combined with an EPO receptor activator, could be used to treat a chronic iron-deficiency.

Myelodysplastic syndrome (MDS) is a diverse collection of hematological conditions characterized by ineffective production of myeloid blood cells and risk of transformation to acute myelogenous leukemia. In MDS patients, blood stem cells do not mature into healthy red blood cells, white blood cells, or platelets. MDS disorders include, for example, refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, refractory cytopenia with multilineage dysplasia, and myelodysplastic syndrome associated with an isolated 5q chromosome abnormality. As these disorders manifest as irreversible defects in both quantity and quality of hematopoietic cells, most MDS patients are afflicted with chronic anemia. Therefore, MDS patients eventually require blood transfusions and/or treatment with growth factors (e.g., erythropoietin or G-CSF) to increase red blood cell levels. However, many MDS patients develop side-effects due to frequency of such therapies. For example, patients who receive frequent red blood cell transfusion can exhibit tissue and organ damage from the buildup of extra iron. Accordingly, one or more TGF-beta superfamily heteromultimers of the disclosure, may be used to treat patients having MDS. In certain embodiments, patients

suffering from MDS may be treated using one or more TGF-beta superfamily heteromultimers of the disclosure, optionally in combination with an EPO receptor activator. In other embodiments, patients suffering from MDS may be treated using a combination of one or more TGF-beta superfamily heteromultimers of the disclosure and one or more additional therapeutic agents for treating MDS including, for example, thalidomide, lenalidomide, azacitadine, decitabine, erythropoietins, deferoxamine, antithymocyte globulin, and filgrastrim (G-CSF).

Originally distinguished from aplastic anemia, hemorrhage, or peripheral hemolysis on the basis of ferrokinetic studies [see, *e.g.*, Ricketts *et al.* (1978) Clin Nucl Med 3:159-164], ineffective erythropoiesis describes a diverse group of anemias in which production of mature RBCs is less than would be expected given the number of erythroid precursors (erythroblasts) present in the bone marrow [Tanno *et al.* (2010) Adv Hematol 2010:358283]. In such anemias, tissue hypoxia persists despite elevated erythropoietin levels due to ineffective production of mature RBCs. A vicious cycle eventually develops in which elevated erythropoietin levels drive massive expansion of erythroblasts, potentially leading to splenomegaly (spleen enlargement) due to extramedullary erythropoiesis [see, *e.g.*, Aizawa *et al.* (2003) Am J Hematol 74:68-72], erythroblast-induced bone pathology [see, *e.g.*, Di Matteo *et al.* (2008) J Biol Regul Homeost Agents 22:211-216], and tissue iron overload, even in the absence of therapeutic RBC transfusions [see, *e.g.*, Pippard *et al.* (1979) Lancet 2:819-821]. Thus, by boosting erythropoietic effectiveness, one or more TGF-beta superfamily heteromultimer complexes of the present disclosure may break the aforementioned cycle and thus alleviate not only the underlying anemia but also the associated complications of elevated erythropoietin levels, splenomegaly, bone pathology, and tissue iron overload. In some embodiments, one or more TGF-beta superfamily heteromultimers of the present disclosure can be used to treat or prevent ineffective erythropoiesis, including anemia and elevated EPO levels as well as complications such as splenomegaly, erythroblast-induced bone pathology, iron overload, and their attendant pathologies. In some embodiments, the elevated EPO levels are relative to one or more healthy control patients of similar age and/or the same sex. With splenomegaly, such pathologies include thoracic or abdominal pain and reticuloendothelial hyperplasia. Extramedullary hematopoiesis can occur not only in the spleen but potentially in other tissues in the form of extramedullary hematopoietic pseudotumors [see, *e.g.*, Musallam *et al.* (2012) Cold Spring Harb Perspect Med 2:a013482]. With erythroblast-induced bone pathology,

attendant pathologies include low bone mineral density, osteoporosis, and bone pain [see, *e.g.*, Haidar *et al.* (2011) Bone 48:425-432]. With iron overload, attendant pathologies include hepcidin suppression and hyperabsorption of dietary iron [see, *e.g.*, Musallam *et al.* (2012) Blood Rev 26(Suppl 1):S16-S19], multiple endocrinopathies and liver fibrosis/cirrhosis [see, *e.g.*, Galanello *et al.* (2010) Orphanet J Rare Dis 5:11], and iron-overload cardiomyopathy [Lekawanvijit *et al.*, 2009, Can J Cardiol 25:213-218].

The most common causes of ineffective erythropoiesis are the thalassemia syndromes, hereditary hemoglobinopathies in which imbalances in the production of intact alpha- and beta-hemoglobin chains lead to increased apoptosis during erythroblast maturation [see, *e.g.*, Schrier (2002) Curr Opin Hematol 9:123-126]. Thalassemias are collectively among the most frequent genetic disorders worldwide, with changing epidemiologic patterns predicted to contribute to a growing public health problem in both the U.S. and globally [Vichinsky (2005) Ann NY Acad Sci 1054:18-24]. Thalassemia syndromes are named according to their severity. Thus, α-thalassemias include α-thalassemia minor (also known as α-thalassemia trait; two affected α-globin genes), hemoglobin H disease (three affected α-globin genes), and α-thalassemia major (also known as hydrops fetalis; four affected α-globin genes). β-Thalassemias include β-thalassemia minor (also known as β-thalassemia trait; one affected β-globin gene), β-thalassemia intermedia (two affected β-globin genes), hemoglobin E thalassemia (two affected β-globin genes), and β-thalassemia major (also known as Cooley's anemia; two affected β-globin genes resulting in a complete absence of β-globin protein). β-Thalassemia impacts multiple organs, is associated with considerable morbidity and mortality, and currently requires life-long care. Although life expectancy in patients with β-thalassemia has increased in recent years due to use of regular blood transfusions in combination with iron chelation, iron overload resulting both from transfusions and from excessive gastrointestinal absorption of iron can cause serious complications such as heart disease, thrombosis, hypogonadism, hypothyroidism, diabetes, osteoporosis, and osteopenia [see, *e.g.*, Rund *et al.* (2005) N Engl J Med 353:1135-1146]. In certain embodiments, one or more TGF-beta superfamily heteromultimers of the disclosure, optionally combined with an EPO receptor activator, can be used to treat or prevent a thalassemia syndrome.

In some embodiments, one or more TGF-beta superfamily heteromultimers of the disclosure, optionally combined with an EPO receptor activator, can be used for treating disorders of ineffective erythropoiesis besides thalassemia syndromes. Such disorders include siderblastic anemia (inherited or acquired); dyserythropoietic anemia (types I and II);

sickle cell anemia; hereditary spherocytosis; pyruvate kinase deficiency; megaloblastic anemias, potentially caused by conditions such as folate deficiency (due to congenital diseases, decreased intake, or increased requirements), cobalamin deficiency (due to congenital diseases, pernicious anemia, impaired absorption, pancreatic insufficiency, or decreased intake), certain drugs, or unexplained causes (congenital dyserythropoietic anemia, refractory megaloblastic anemia, or erythroleukemia); myelophthisic anemias including, for example, myelofibrosis (myeloid metaplasia) and myelophthisis; congenital erythropoietic porphyria; and lead poisoning.

In certain embodiments, one or more TGF-beta superfamily heteromultimers of the disclosure may be used in combination with supportive therapies for ineffective erythropoiesis. Such therapies include transfusion with either red blood cells or whole blood to treat anemia. In chronic or hereditary anemias, normal mechanisms for iron homeostasis are overwhelmed by repeated transfusions, eventually leading to toxic and potentially fatal accumulation of iron in vital tissues such as heart, liver, and endocrine glands. Thus, supportive therapies for patients chronically afflicted with ineffective erythropoiesis also include treatment with one or more iron-chelating molecules to promote iron excretion in the urine and/or stool and thereby prevent, or reverse, tissue iron overload [see, *e.g.*, Hershko (2006) Haematologica 91:1307-1312; Cao *et al.* (2011), Pediatr Rep 3(2):e17]. Effective iron-chelating agents should be able to selectively bind and neutralize ferric iron, the oxidized form of non-transferrin bound iron which likely accounts for most iron toxicity through catalytic production of hydroxyl radicals and oxidation products [see, *e.g.*, Esposito et al. (2003) Blood 102:2670-2677]. These agents are structurally diverse, but all possess oxygen or nitrogen donor atoms able to form neutralizing octahedral coordination complexes with individual iron atoms in stoichiometries of 1:1 (hexadentate agents), 2:1 (tridentate), or 3:1 (bidentate) [Kalinowski *et al.* (2005) Pharmacol Rev 57:547-583]. In general, effective iron-chelating agents also are relatively low molecular weight (*e.g.*, less than 700 daltons), with solubility in both water and lipids to enable access to affected tissues. Specific examples of iron-chelating molecules include deferoxamine, a hexadentate agent of bacterial origin requiring daily parenteral administration, and the orally active synthetic agents deferiprone (bidentate) and deferasirox (tridentate). Combination therapy consisting of same-day administration of two iron-chelating agents shows promise in patients unresponsive to chelation monotherapy and also in overcoming issues of poor patient compliance with

dereroxamine alone [Cao *et al.* (2011) Pediatr Rep 3(2):e17; Galanello *et al.* (2010) Ann NY Acad Sci 1202:79-86].

In certain aspects, one or more TGF-beta superfamily heteromultimers of the disclosure may be used to decrease blood cell transfusion burden in a patient. For example, a TGF-beta superfamily heteromultimer may be used to decrease blood cell transfusion by greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% for 4 to 8 weeks relative to the equal time prior to the start of the TGF-beta superfamily heteromultimer treatment. In some embodiments, a TGF-beta superfamily heteromultimer may be used to decrease blood cell transfusion by greater than about 50% for 4 to 8 weeks relative to the equal time prior to the start of the TGF-beta superfamily heteromultimer treatment in a patient. In certain embodiments, a patient may be treated with a dosing regimen intended to restore the patient to a target level of red blood cells, hemoglobin, and/or hematocrit or allow the reduction or elimination of red blood cell transfusions (reduce transfusion burden) while maintaining an acceptable level of red blood cells, hemoglobin and/or hematocrit. As hemoglobin and hematocrit levels vary from person to person, optimally, the target hemoglobin and/or hematocrit level can be individualized for each patient.

As used herein, "in combination with" or "conjoint administration" refers to any form of administration such that the second therapy is still effective in the body (e.g., the two compounds are simultaneously effective in the patient, which may include synergistic effects of the two compounds). Effectiveness may not correlate to measurable concentration of the agent in blood, serum, or plasma. For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially, and on different schedules. Thus, an individual who receives such treatment can benefit from a combined effect of different therapies. One or more TGF-beta superfamily heteromultimers of the disclosure can be administered concurrently with, prior to, or subsequent to, one or more other additional agents or supportive therapies. In general, each therapeutic agent will be administered at a dose and/or on a time schedule determined for that particular agent. The particular combination to employ in a regimen will take into account compatibility of the antagonist of the present disclosure with the therapy and/or the desired therapeutic effect to be achieved.

In certain embodiments, one or more TGF-beta superfamily heteromultimers of the disclosure may be used in combination with hepcidin or a hepcidin agonist for ineffective

erythropoiesis. A circulating polypeptide produced mainly in the liver, hepcidin is considered a master regulator of iron metabolism by virtue of its ability to induce the degradation of ferroportin, an iron-export protein localized on absorptive enterocytes, hepatocytes, and macrophages. Broadly speaking, hepcidin reduces availability of extracellular iron, so hepcidin agonists may be beneficial in the treatment of ineffective erythropoiesis [see, *e.g.*, Nemeth (2010) Adv Hematol 2010:750643]. This view is supported by beneficial effects of increased hepcidin expression in a mouse model of β-thalassemia [Gardenghi *et al.* (2010) J Clin Invest 120:4466-4477].

One or more TGF-beta superfamily heteromultimers of the disclosure, optionally combined with an EPO receptor activator, would also be appropriate for treating anemias of disordered RBC maturation, which are characterized in part by undersized (microcytic), oversized (macrocytic), misshapen, or abnormally colored (hypochromic) RBCs.

In certain embodiments, the present disclosure provides methods of treating or preventing anemia in an individual in need thereof by administering to the individual a therapeutically effective amount of one or more TGF-beta superfamily heteromultimers of the disclosure and a EPO receptor activator. In certain embodiments, one or more TGF-beta superfamily heteromultimers of the disclosure may be used in combination with EPO receptor activators to reduce the required dose of these activators in patients that are susceptible to adverse effects of EPO. These methods may be used for therapeutic and prophylactic treatments of a patient.

One or more TGF-beta superfamily heteromultimers of the disclosure may be used in combination with EPO receptor activators to achieve an increase in red blood cells, particularly at lower dose ranges of EPO receptor activators. This may be beneficial in reducing the known off-target effects and risks associated with high doses of EPO receptor activators. The primary adverse effects of EPO include, for example, an excessive increase in the hematocrit or hemoglobin levels and polycythemia. Elevated hematocrit levels can lead to hypertension (more particularly aggravation of hypertension) and vascular thrombosis. Other adverse effects of EPO which have been reported, some of which relate to hypertension, are headaches, influenza-like syndrome, obstruction of shunts, myocardial infarctions and cerebral convulsions due to thrombosis, hypertensive encephalopathy, and red cell blood cell aplasia. See, *e.g.*, Singibarti (1994) J. Clin Investig 72(suppl 6), S36-S43; Horl *et al.* (2000) Nephrol Dial Transplant 15(suppl 4), 51-56; Delanty *et al.* (1997) Neurology 49, 686-689; and Bunn (2002) N Engl J Med 346(7), 522-523).

Provided that TGF-beta superfamily heteromultimers of the present disclosure act by a different mechanism than EPO, these antagonists may be useful for increasing red blood cell and hemoglobin levels in patients that do not respond well to EPO. For example, a TGF-beta superfamily heteromultimer of the present disclosure may be beneficial for a patient in which administration of a normal-to-increased dose of EPO (>300 IU/kg/week) does not result in the increase of hemoglobin level up to the target level. Patients with an inadequate EPO response are found in all types of anemia, but higher numbers of non-responders have been observed particularly frequently in patients with cancers and patients with end-stage renal disease. An inadequate response to EPO can be either constitutive (observed upon the first treatment with EPO) or acquired (observed upon repeated treatment with EPO).

In certain embodiments, the present disclosure provides methods for managing a patient that has been treated with, or is a candidate to be treated with, one or more TGF-beta superfamily heteromultimers of the disclosure by measuring one or more hematologic parameters in the patient. The hematologic parameters may be used to evaluate appropriate dosing for a patient who is a candidate to be treated with the antagonist of the present disclosure, to monitor the hematologic parameters during treatment, to evaluate whether to adjust the dosage during treatment with one or more antagonist of the disclosure, and/or to evaluate an appropriate maintenance dose of one or more antagonists of the disclosure. If one or more of the hematologic parameters are outside the normal level, dosing with one or more TGF-beta superfamily heteromultimers of the disclosure may be reduced, delayed or terminated.

Hematologic parameters that may be measured in accordance with the methods provided herein include, for example, red blood cell levels, blood pressure, iron stores, and other agents found in bodily fluids that correlate with increased red blood cell levels, using art-recognized methods. Such parameters may be determined using a blood sample from a patient. Increases in red blood cell levels, hemoglobin levels, and/or hematocrit levels may cause increases in blood pressure.

In one embodiment, if one or more hematologic parameters are outside the normal range or on the high side of normal in a patient who is a candidate to be treated with one or more TGF-beta superfamily heteromultimers of the disclosure, then onset of administration of the one or more TGF-beta superfamily heteromultimers of the disclosure may be delayed until the hematologic parameters have returned to a normal or acceptable level either naturally or via therapeutic intervention. For example, if a candidate patient is hypertensive

or pre-hypertensive, then the patient may be treated with a blood pressure lowering agent in order to reduce the patient's blood pressure. Any blood pressure lowering agent appropriate for the individual patient's condition may be used including, for example, diuretics, adrenergic inhibitors (including alpha blockers and beta blockers), vasodilators, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, or angiotensin II receptor blockers. Blood pressure may alternatively be treated using a diet and exercise regimen. Similarly, if a candidate patient has iron stores that are lower than normal, or on the low side of normal, then the patient may be treated with an appropriate regimen of diet and/or iron supplements until the patient's iron stores have returned to a normal or acceptable level. For patients having higher than normal red blood cell levels and/or hemoglobin levels, then administration of the one or more TGF-beta superfamily heteromultimers of the disclosure may be delayed until the levels have returned to a normal or acceptable level.

In certain embodiments, if one or more hematologic parameters are outside the normal range or on the high side of normal in a patient who is a candidate to be treated with one or more TGF-beta superfamily heteromultimers of the disclosure, then the onset of administration may not be delayed. However, the dosage amount or frequency of dosing of the one or more TGF-beta superfamily heteromultimers of the disclosure may be set at an amount that would reduce the risk of an unacceptable increase in the hematologic parameters arising upon administration of the one or more TGF-beta superfamily heteromultimers of the disclosure. Alternatively, a therapeutic regimen may be developed for the patient that combines one or more TGF-beta superfamily heteromultimers of the disclosure with a therapeutic agent that addresses the undesirable level of the hematologic parameter. For example, if the patient has elevated blood pressure, then a therapeutic regimen involving administration of one or more TGF-beta superfamily heteromultimers of the disclosure and a blood pressure-lowering agent may be designed. For a patient having lower than desired iron stores, a therapeutic regimen of one or more TGF-beta superfamily heteromultimers of the disclosure and iron supplementation may be developed.

In one embodiment, baseline parameter(s) for one or more hematologic parameters may be established for a patient who is a candidate to be treated with one or more TGF-beta superfamily heteromultimers of the disclosure and an appropriate dosing regimen established for that patient based on the baseline value(s). Alternatively, established baseline parameters based on a patient's medical history could be used to inform an appropriate dosing regimen for a patient. For example, if a healthy patient has an established baseline blood pressure

reading that is above the defined normal range it may not be necessary to bring the patient's blood pressure into the range that is considered normal for the general population prior to treatment with the one or more TGF-beta superfamily heteromultimers of the disclosure. A patient's baseline values for one or more hematologic parameters prior to treatment with one or more TGF-beta superfamily heteromultimers of the disclosure may also be used as the relevant comparative values for monitoring any changes to the hematologic parameters during treatment with the one or more TGF-beta superfamily heteromultimers of the disclosure.

In certain embodiments, one or more hematologic parameters are measured in patients who are being treated with a one or more TGF-beta superfamily heteromultimers of the disclosure. The hematologic parameters may be used to monitor the patient during treatment and permit adjustment or termination of the dosing with the one or more TGF-beta superfamily heteromultimers of the disclosure or additional dosing with another therapeutic agent. For example, if administration of one or more TGF-beta superfamily heteromultimers of the disclosure of the disclosure results in an increase in blood pressure, red blood cell level, or hemoglobin level, or a reduction in iron stores, then the dose of the one or more TGF-beta superfamily heteromultimers of the disclosure may be reduced in amount or frequency in order to decrease the effects of the one or more TGF-beta superfamily heteromultimers of the disclosure on the one or more hematologic parameters. If administration of one or more TGF-beta superfamily heteromultimers of the disclosure results in a change in one or more hematologic parameters that is adverse to the patient, then the dosing of the one or more TGF-beta superfamily heteromultimers of the disclosure may be terminated either temporarily, until the hematologic parameter(s) return to an acceptable level, or permanently. Similarly, if one or more hematologic parameters are not brought within an acceptable range after reducing the dose or frequency of administration of the one or more TGF-beta superfamily heteromultimers of the disclosure, then the dosing may be terminated. As an alternative, or in addition to, reducing or terminating the dosing with the one or more TGF-beta superfamily heteromultimers of the disclosure, the patient may be dosed with an additional therapeutic agent that addresses the undesirable level in the hematologic parameter(s), such as, for example, a blood pressure-lowering agent or an iron supplement. For example, if a patient being treated with one or more TGF-beta superfamily heteromultimers of the disclosure has elevated blood pressure, then dosing with the one or more TGF-beta superfamily heteromultimers of the disclosure may continue at the same level

and a blood pressure-lowering agent is added to the treatment regimen, dosing with the one or more TGF-beta superfamily heteromultimers of the disclosure may be reduced (*e.g.*, in amount and/or frequency) and a blood pressure-lowering agent is added to the treatment regimen, or dosing with the one or more TGF-beta superfamily heteromultimers of the disclosure may be terminated and the patient may be treated with a blood pressure-lowering agent.

6. Pharmaceutical Compositions

In certain aspects, TGF-beta superfamily heteromultimers of the present disclosure can be administered alone or as a component of a pharmaceutical formulation (also referred to as a therapeutic composition or pharmaceutical composition). A pharmaceutical formation refers to a preparation which is in such form as to permit the biological activity of an active ingredient (*e.g.*, an agent of the present disclosure) contained therein to be effective and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. The subject compounds may be formulated for administration in any convenient way for use in human or veterinary medicine. For example, one or more agents of the present disclosure may be formulated with a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is generally nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, and/or preservative. In some embodiments, pharmaceutical formulations for use in the present disclosure are in a pyrogen-free, physiologically-acceptable form when administered to a subject. Therapeutically useful agents other than those described herein, which may optionally be included in the formulation as described above, may be administered in combination with the subject agents in the methods of the present disclosure.

In certain embodiments, compositions will be administered parenterally [*e.g.*, by intravenous (I.V.) injection, intraarterial injection, intraosseous injection, intramuscular injection, intrathecal injection, subcutaneous injection, or intradermal injection]. Pharmaceutical compositions suitable for parenteral administration may comprise one or more agents of the disclosure in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just

prior to use. Injectable solutions or dispersions may contain antioxidants, buffers, bacteriostats, suspending agents, thickening agents, or solutes which render the formulation isotonic with the blood of the intended recipient. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical formulations of the present disclosure include water, ethanol, polyols (*e.g.*, glycerol, propylene glycol, polyethylene glycol, *etc.*), vegetable oils (*e.g.*, olive oil), injectable organic esters (*e.g.*, ethyl oleate), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by the use of coating materials (*e.g.*, lecithin), by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

In some embodiments, a therapeutic method of the present disclosure includes administering the pharmaceutical composition systemically, or locally, from an implant or device. Further, the pharmaceutical composition may be encapsulated or injected in a form for delivery to a target tissue site (*e.g.*, bone marrow or muscle). In certain embodiments, compositions of the present disclosure may include a matrix capable of delivering one or more of the agents of the present disclosure to a target tissue site (*e.g.*, bone marrow or muscle), providing a structure for the developing tissue and optimally capable of being resorbed into the body. For example, the matrix may provide slow release of one or more agents of the present disclosure. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material may be based on one or more of: biocompatibility, biodegradability, mechanical properties, cosmetic appearance, and interface properties. The particular application of the subject compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, and polyanhydrides. Other potential materials are biodegradable and biologically well-defined including, for example, bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are non-biodegradable and chemically defined including, for example, sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material including, for example, polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition (*e.g.*, calcium-aluminate-phosphate) and processing to alter one or more of pore size, particle size, particle shape, and biodegradability.

In certain embodiments, pharmaceutical compositions of present disclosure can be administered topically. "Topical application" or "topically" means contact of the pharmaceutical composition with body surfaces including, for example, the skin, wound sites, and mucous membranes. The topical pharmaceutical compositions can have various application forms and typically comprises a drug-containing layer, which is adapted to be placed near to or in direct contact with the tissue upon topically administering the composition. Pharmaceutical compositions suitable for topical administration may comprise one or more one or more TGFβ superfamily type I and/or type II receptor polypeptide heteromultimers of the disclosure in combination formulated as a liquid, a gel, a cream, a lotion, an ointment, a foam, a paste, a putty, a semi-solid, or a solid. Compositions in the liquid, gel, cream, lotion, ointment, foam, paste, or putty form can be applied by spreading, spraying, smearing, dabbing or rolling the composition on the target tissue. The compositions also may be impregnated into sterile dressings, transdermal patches, plasters, and bandages. Compositions of the putty, semi-solid or solid forms may be deformable. They may be elastic or non-elastic (*e.g.*, flexible or rigid). In certain aspects, the composition forms part of a composite and can include fibers, particulates, or multiple layers with the same or different compositions.

Topical compositions in the liquid form may include pharmaceutically acceptable solutions, emulsions, microemulsions, and suspensions. In addition to the active ingredient(s), the liquid dosage form may contain an inert diluent commonly used in the art including, for example, water or other solvent, a solubilizing agent and/or emulsifier [*e.g.*, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, or 1,3-butylene glycol, an oil (*e.g.*, cottonseed, groundnut, corn, germ, olive, castor, and sesame oil), glycerol, tetrahydrofuryl alcohol, a polyethylene glycol, a fatty acid ester of sorbitan, and mixtures thereof].

Topical gel, cream, lotion, ointment, semi-solid or solid compositions may include one or more thickening agents, such as a polysaccharide, synthetic polymer or protein-based polymer. In one embodiment of the invention, the gelling agent herein is one that is suitably nontoxic and gives the desired viscosity. The thickening agents may include polymers, copolymers, and monomers of: vinylpyrrolidones, methacrylamides, acrylamides N-vinylimidazoles, carboxy vinyls, vinyl esters, vinyl ethers, silicones, polyethyleneoxides, polyethyleneglycols, vinylalcohols, sodium acrylates, acrylates, maleic acids, NN-dimethylacrylamides, diacetone acrylamides, acrylamides, acryloyl morpholine, pluronic,

collagens, polyacrylamides, polyacrylates, polyvinyl alcohols, polyvinylenes, polyvinyl silicates, polyacrylates substituted with a sugar (e.g., sucrose, glucose, glucosamines, galactose, trehalose, mannose, or lactose), acylamidopropane sulfonic acids, tetramethoxyorthosilicates, methyltrimethoxyorthosilicates, tetraalkoxyorthosilicates, trialkoxyorthosilicates, glycols, propylene glycol, glycerine, polysaccharides, alginates, dextrans, cyclodextrin, celluloses, modified celluloses, oxidized celluloses, chitosans, chitins, guars, carrageenans, hyaluronic acids, inulin, starches, modified starches, agarose, methylcelluloses, plant gums, hylaronans, hydrogels, gelatins, glycosaminoglycans, carboxymethyl celluloses, hydroxyethyl celluloses, hydroxy propyl methyl celluloses, pectins, low-methoxy pectins, cross-linked dextrans, starch-acrylonitrile graft copolymers, starch sodium polyacrylate, hydroxyethyl methacrylates, hydroxyl ethyl acrylates, polyvinylene, polyethylvinylethers, polymethyl methacrylates, polystyrenes, polyurethanes, polyalkanoates, polylactic acids, polylactates, poly(3-hydroxybutyrate), sulfonated hydrogels, AMPS (2-acrylamido-2-methyl-1-propanesulfonic acid), SEM (sulfoethylmethacrylate), SPM (sulfopropyl methacrylate), SPA (sulfopropyl acrylate), N,N-dimethyl-N-methacryloxyethyl-N-(3-sulfopropyl)ammonium betaine, methacryllic acid amidopropyl-dimethyl ammonium sulfobetaine, SPI (itaconic acid-bis(1-propyl sulfonizacid-3) ester di-potassium salt), itaconic acids, AMBC (3-acrylamido-3-methylbutanoic acid), beta-carboxyethyl acrylate (acrylic acid dimers), and maleic anhydride-methylvinyl ether polymers, derivatives thereof, salts thereof, acids thereof, and combinations thereof. In certain embodiments, pharmaceutical compositions of present disclosure can be administered orally, for example, in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis such as sucrose and acacia or tragacanth), powders, granules, a solution or a suspension in an aqueous or non-aqueous liquid, an oil-in-water or water-in-oil liquid emulsion, or an elixir or syrup, or pastille (using an inert base, such as gelatin and glycerin, or sucrose and acacia), and/or a mouth wash, each containing a predetermined amount of a compound of the present disclosure and optionally one or more other active ingredients. A compound of the present disclosure and optionally one or more other active ingredients may also be administered as a bolus, electuary, or paste.

In solid dosage forms for oral administration (*e.g.*, capsules, tablets, pills, dragees, powders, and granules), one or more compounds of the present disclosure may be mixed with one or more pharmaceutically acceptable carriers including, for example, sodium citrate, dicalcium phosphate, a filler or extender (*e.g.*, a starch, lactose, sucrose, glucose, mannitol, and silicic acid), a binder (*e.g.* carboxymethylcellulose, an alginate, gelatin, polyvinyl

pyrrolidone, sucrose, and acacia), a humectant (*e.g.*, glycerol), a disintegrating agent (*e.g.*, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, a silicate, and sodium carbonate), a solution retarding agent (*e.g.* paraffin), an absorption accelerator (*e.g.* a quaternary ammonium compound), a wetting agent (*e.g.*, cetyl alcohol and glycerol monostearate), an absorbent (*e.g.*, kaolin and bentonite clay), a lubricant (*e.g.*, a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), a coloring agent, and mixtures thereof. In the case of capsules, tablets, and pills, the pharmaceutical formulation (composition) may also comprise a buffering agent. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using one or more excipients including, e.g., lactose or a milk sugar as well as a high molecular-weight polyethylene glycol.

Liquid dosage forms for oral administration of the pharmaceutical composition may include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient(s), the liquid dosage form may contain an inert diluent commonly used in the art including, for example, water or other solvent, a solubilizing agent and/or emulsifier [*e.g.*, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, or 1,3-butylene glycol, an oil (*e.g.*, cottonseed, groundnut, corn, germ, olive, castor, and sesame oil), glycerol, tetrahydrofuryl alcohol, a polyethylene glycol, a fatty acid ester of sorbitan, and mixtures thereof]. Besides inert diluents, the oral formulation can also include an adjuvant including, for example, a wetting agent, an emulsifying and suspending agent, a sweetening agent, a flavoring agent, a coloring agent, a perfuming agent, a preservative agent, and combinations thereof.

Suspensions, in addition to the active compounds, may contain suspending agents including, for example, an ethoxylated isostearyl alcohol, polyoxyethylene sorbitol, a sorbitan ester, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth, and combinations thereof.

Prevention of the action and/or growth of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents including, for example, paraben, chlorobutanol, and phenol sorbic acid.

In certain embodiments, it may be desirable to include an isotonic agent including, for example, a sugar or sodium chloride into the compositions. In addition, prolonged absorption

of an injectable pharmaceutical form may be brought about by the inclusion of an agent that delay absorption including, for example, aluminum monostearate and gelatin.

It is understood that the dosage regimen will be determined by the attending physician considering various factors which modify the action of the one or more of the agents of the present disclosure. In the case of a TGF-beta superfamily heteromultimer that promotes red blood cell formation, various factors may include, but are not limited to, the patient's red blood cell count, hemoglobin level, the desired target red blood cell count, the patient's age, the patient's sex, the patient's diet, the severity of any disease that may be contributing to a depressed red blood cell level, the time of administration, and other clinical factors. The addition of other known active agents to the final composition may also affect the dosage. Progress can be monitored by periodic assessment of one or more of red blood cell levels, hemoglobin levels, reticulocyte levels, and other indicators of the hematopoietic process.

In certain embodiments, the present disclosure also provides gene therapy for the *in vivo* production of one or more of the agents of the present disclosure. Such therapy would achieve its therapeutic effect by introduction of the agent sequences into cells or tissues having one or more of the disorders as listed above. Delivery of the agent sequences can be achieved, for example, by using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Preferred therapeutic delivery of one or more of agent sequences of the disclosure is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or an RNA virus (*e.g.*, a retrovirus). The retroviral vector may be a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. Retroviral vectors can be made target-specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody. Those of skill in the art will recognize that specific polynucleotide sequences can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing one or more of the agents of the present disclosure.

Alternatively, tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes (gag, pol, and env), by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for one or more of the agents of the present disclosure is a colloidal dispersion system. Colloidal dispersion systems include, for example, macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. In certain embodiments, the preferred colloidal system of this disclosure is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. RNA, DNA, and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form. See, *e.g.*, Fraley, et al. (1981) Trends Biochem. Sci., 6:77. Methods for efficient gene transfer using a liposome vehicle are known in the art. See, *e.g.*, Mannino, *et al.* (1988) Biotechniques, 6:682, 1988.

The composition of the liposome is usually a combination of phospholipids, which may include a steroid (*e.g.*cholesterol). The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations. Other phospholipids or other lipids may also be used including, for example a phosphatidyl compound (*e.g.*, phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, a sphingolipid, a cerebroside, and a ganglioside), egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine. The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art.

EXEMPLIFICATION

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain embodiments and embodiments of the present invention, and are not intended to limit the invention.

Example 1. Generation of an ActRIIB-Fc:ALK4-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK4-Fc heteromultimer comprising the extracellular domains of human ActRIIB and human ALK4, which are each separately

fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

A methodology for promoting formation of ActRIIB-Fc:ALK4-Fc heteromultimers, as opposed to the ActRIIB-Fc or ALK4-Fc homomultimer, is to introduce alterations in the amino acid sequence of the Fc domains to guide the formation of asymmetric heteromeric complexes. Many different approaches to making asymmetric interaction pairs using Fc domains are described in this disclosure.

In one approach, illustrated in the ActRIIB-Fc and ALK4-Fc polypeptide sequences of SEQ ID NOs: 100-102 and 104-106, respectively, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide each employ the tissue plasminogen activator (TPA) leader: MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 98).

The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 100) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPSRKEMT KNQVSLTCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK          (SEQ ID NO: 100)
```

The leader (signal) sequence and linker are underlined. To promote formation of the ActRIIB-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the ActRIIB fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 100 may optionally be provided with lysine (K) removed from the C-terminus.

This ActRIIB-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 101):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
 101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
 151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
 201  CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
 251  GGCTAGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
 301  GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
 351  GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
 401  CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
 451  CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
 501  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
 551  TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 601  GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
 651  CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
 701  GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
 751  GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
 801  ACAGGTGTAC ACCCTGCCCC CATCCCGGAA GGAGATGACC AAGAACCAGG
 851  TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
 951  CGTGCTGAAG TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
1101  TAAA     (SEQ ID NO: 101)
```

The mature ActRIIB-Fc fusion polypeptide (SEQ ID NO: 102) is as follows, and may optionally be provided with lysine removed from the C-terminus.

```
   1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
  51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
 101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
 151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
 201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
 251  RKEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLKSDGSF
 301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
      (SEQ ID NO: 102)
```

The complementary form of ALK4-Fc fusion polypeptide (SEQ ID NO: 104) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 51  GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101  YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
301  DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPG      (SEQ ID NO: 104)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs: 100 and 102 above, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK4-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 104 may optionally be provided with lysine added at the C-terminus.

This ALK4-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 105):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCCGGGCC CCGGGGGGTC CAGGCTCTGC
 101  TGTGTGCGTG CACCAGCTGC CTCCAGGCCA ACTACACGTG TGAGACAGAT
 151  GGGGCCTGCA TGGTTTCCAT TTTCAATCTG GATGGGATGG AGCACCATGT
 201  GCGCACCTGC ATCCCCAAAG TGGAGCTGGT CCCTGCCGGG AAGCCCTTCT
 251  ACTGCCTGAG CTCGGAGGAC CTGCGCAACA CCCACTGCTG CTACACTGAC
 301  TACTGCAACA GGATCGACTT GAGGGTGCCC AGTGGTCACC TCAAGGAGCC
 351  TGAGCACCCG TCCATGTGGG GCCCGGTGGA GACCGGTGGT GGAACTCACA
 401  CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
 451  CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
 501  GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
 551  TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
 601  CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
 651  CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA
 701  ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
 751  CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGAGGAGAT
 801  GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA
```

```
 851  GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC
 901  GACACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTATAG
 951  CGACCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001  GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
1051  TCCCTGTCTC CGGGT      (SEQ ID NO:105)
```

The mature ALK4-Fc fusion protein sequence (SEQ ID NO: 106) is as follows and may optionally be provided with lysine added at the C-terminus.

```
  1 SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
 51 ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101 PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
251 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF LYSDLTVDKS
301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G   (SEQ ID NO: 106)
```

The ActRIIB-Fc and ALK4-Fc proteins of SEQ ID NO: 102 and SEQ ID NO: 106, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK4-Fc.

In another approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ActRIIB-Fc and ALK4-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 403-404, respectively. The ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide each employ the tissue plasminogen activator (TPA) leader: MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 98).

The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 401) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK          (SEQ ID NO: 401)
```

The leader (signal) sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK4-Fc heterodimer rather than either of the possible

homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 401 may optionally be provided with lysine removed from the C-terminus.

The mature ActRIIB-Fc fusion polypeptide is as follows:

```
  1   GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51   IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101   GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151   RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201   VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC
251   REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301   FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
          (SEQ ID NO:402)
```

The complementary form of ALK4-Fc fusion polypeptide (SEQ ID NO: 403) is as follows and may optionally be provided with lysine removed from the C-terminus.

```
  1   MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 51   GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101   YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151   LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201   REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251   QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY
301   KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351   SLSPGK    (SEQ ID NO: 403)
```

The leader sequence and the linker are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs: 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK4 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 403 may optionally be provided with lysine removed from the C-terminus.

The mature ALK4-Fc fusion protein sequence is as follows and may optionally be provided with lysine removed from the C-terminus.

```
  1   SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
 51   ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101   PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151   VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201   GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
```

251 SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS
301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 404)

The ActRIIB-Fc and ALK4-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 404, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK4-Fc.

Purification of various ActRIIB-Fc:ALK4-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

In another approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions, an additional intermolecular disulfide bond, and electrostatic differences for facilitating purification, as illustrated in the ActRIIB-Fc and ALK4-Fc polypeptide sequences of SEQ ID NOs: 700-730 and 740-770, respectively. The ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide each employ the tissue plasminogen activator (TPA) leader.

The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 700) is shown below:

1 MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101 ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251 APIEKTISKA KGQPREPQVY TLPPCREEMT ENQVSLWCLV KGFYPSDIAV
301 EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQD SLSLSPG (SEQ ID NO: 700)

The leader sequence and linker are underlined. To promote formation of the ALK4-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline

above. To facilitate purification of the ALK4-Fc:ActRIIB-Fc heterodimer, two amino acid substitutions (replacing lysines with acidic amino acids) can also be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 700 may optionally be provided with a lysine added at the C-terminus.

This ActRIIB-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 710):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
 101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
 151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
 201  CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
 251  GGCTAGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
 301  GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
 351  GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
 401  CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
 451  CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
 501  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
 551  TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 601  GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
 651  CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
 701  GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
 751  GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
 801  ACAGGTGTAC ACCCTGCCCC CATGCCGGGA GGAGATGACC GAGAACCAGG
 851  TCAGCCTGTG GTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
 951  CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCACTA CACGCAGGAC AGCCTCTCCC TGTCTCCGGG
1101  T       (SEQ ID NO: 710)
```

The mature ActRIIB-Fc fusion polypeptide is as follows (SEQ ID NO: 720) and may optionally be provided with a lysine added to the C-terminus.

```
   1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
```

```
  51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
 101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
 151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
 201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC
 251  REEMTENQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
 301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQDSLSLS PG
      (SEQ ID NO: 720)
```

This ActRIIB-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 730):

```
   1  GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
  51  GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
 101  AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
 151  ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
 201  TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
 251  GCTGTGAAGG CAACTTCTGC AACGAGCGCT TCACTCATTT GCCAGAGGCT
 301  GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACCGGTGG
 351  TGGAACTCAC ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGGGGAC
 401  CGTCAGTCTT CCTCTTCCCC CCAAAACCCA AGGACACCCT CATGATCTCC
 451  CGGACCCCTG AGGTCACATG CGTGGTGGTG GACGTGAGCC ACGAAGACCC
 501  TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG CATAATGCCA
 551  AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
 601  GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG
 651  CAAGGTCTCC AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA
 701  AAGCCAAAGG GCAGCCCCGA GAACCACAGG TGTACACCCT GCCCCCATGC
 751  CGGGAGGAGA TGACCGAGAA CCAGGTCAGC CTGTGGTGCC TGGTCAAAGG
 801  CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG
 851  AGAACAACTA CAAGACCACG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC
 901  TTCCTCTATA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA
 951  CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC
1001  AGGACAGCCT CTCCCTGTCT CCGGGT      (SEQ ID NO: 730)
```

The complementary form of ALK4-Fc fusion polypeptide (SEQ ID NO: 740) is as follows and may optionally be provided with lysine removed from the C-terminus.

```
   1  MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
```

```
  51  GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
 101  YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
 151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
 201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
 251  QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESRGQPENNY
 301  KTTPPVLDSR GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
 351  SLSPGK       (SEQ ID NO: 740)
```

The leader sequence and the linker are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs: 700 and 720 above, four amino acid substitutions (replacing a tyrosine with a cysteine, a threonine with a serine, a leucine with an alanine, and a tyrosine with a valine) can be introduced into the Fc domain of the ALK4 fusion polypeptide as indicated by double underline above. To facilitate purification of the ALK4-Fc:ActRIIB-Fc heterodimer, two amino acid substitutions (replacing an asparagine with an arginine and an aspartate with an arginine) can also be introduced into the Fc domain of the ALK4-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 740 may optionally be provided with lysine removed from the C-terminus.

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 750):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCCGGGCC CCGGGGGGTC CAGGCTCTGC
 101  TGTGTGCGTG CACCAGCTGC CTCCAGGCCA ACTACACGTG TGAGACAGAT
 151  GGGGCCTGCA TGGTTTCCAT TTTCAATCTG GATGGGATGG AGCACCATGT
 201  GCGCACCTGC ATCCCCAAAG TGGAGCTGGT CCCTGCCGGG AAGCCCTTCT
 251  ACTGCCTGAG CTCGGAGGAC CTGCGCAACA CCCACTGCTG CTACACTGAC
 301  TACTGCAACA GGATCGACTT GAGGGTGCCC AGTGGTCACC TCAAGGAGCC
 351  TGAGCACCCG TCCATGTGGG GCCCGGTGGA GACCGGTGGT GGAACTCACA
 401  CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
 451  CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
 501  GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
 551  TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
 601  CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
 651  CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA
```

```
 701  ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
 751  CAGCCCCGAG AACCACAGGT GTGCACCCTG CCCCCATCCC GGGAGGAGAT
 801  GACCAAGAAC CAGGTCAGCC TGTCCTGCGC CGTCAAAGGC TTCTATCCCA
 851  GCGACATCGC CGTGGAGTGG GAGAGCCGCG GGCAGCCGGA GAACAACTAC
 901  AAGACCACGC CTCCCGTGCT GGACTCCCGC GGCTCCTTCT TCCTCGTGAG
 951  CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001  GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
1051  TCCCTGTCTC CGGGTAAA        (SEQ ID NO: 750)
```

The mature ALK4-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 760) and may optionally be provided with lysine removed from the C-terminus.

```
   1  SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
  51  ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
 101  PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
 251  SCAVKGFYPS DIAVEWESRG QPENNYKTTP PVLDSRGSFF LVSKLTVDKS
 301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 760)
```

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 770):

```
   1  TCCGGGCCCC GGGGGGTCCA GGCTCTGCTG TGTGCGTGCA CCAGCTGCCT
  51  CCAGGCCAAC TACACGTGTG AGACAGATGG GGCCTGCATG GTTTCCATTT
 101  TCAATCTGGA TGGGATGGAG CACCATGTGC GCACCTGCAT CCCCAAAGTG
 151  GAGCTGGTCC CTGCCGGGAA GCCCTTCTAC TGCCTGAGCT CGGAGGACCT
 201  GCGCAACACC CACTGCTGCT ACACTGACTA CTGCAACAGG ATCGACTTGA
 251  GGGTGCCCAG TGGTCACCTC AAGGAGCCTG AGCACCCGTC CATGTGGGGC
 301  CCGGTGGAGA CCGGTGGTGG AACTCACACA TGCCCACCGT GCCCAGCACC
 351  TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
 401  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
 451  GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT
 501  GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA
 551  CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT
 601  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT
 651  CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
```

```
701  GCACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
751  TCCTGCGCCG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA
801  GAGCCGCGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG
851  ACTCCCGCGG CTCCTTCTTC CTCGTGAGCA AGCTCACCGT GGACAAGAGC
901  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
951  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
     (SEQ ID NO: 770)
```

ActRIIB-Fc and ALK4-Fc proteins of SEQ ID NO: 720 and SEQ ID NO: 760, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ALK4-Fc:ActRIIB-Fc.

In another approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions, an additional intermolecular disulfide bond, and an arginine substitution specifically in the ActRIIB-Fc polypeptide chain for facilitating purification, as illustrated in the ActRIIB-Fc polypeptide sequences of SEQ ID NOs: 780, 790, 800 and 810 and the ALK4-Fc polypeptide sequences of SEQ ID NOs: 480, 820, and 830. The ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide each employ the tissue plasminogen activator (TPA) leader.j

The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 780) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNRYTQK SLSLSPGK          (SEQ ID NO: 780)
```

The leader sequence and linker are underlined. To promote formation of the ALK4-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the ActRIIB-Fc fusion polypeptide as indicated by double underline above. Another amino acid substitution (replacing histidine with arginine)

can also be introduced into the Fc domain of the fusion protein as indicated by double underline above to facilitate purification of the ALK4-Fc:ActRIIB-Fc heterodimer. The amino acid sequence of SEQ ID NO: 780 may optionally be provided with lysine removed from the C-terminus.

This ActRIIB-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 790):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
 101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
 151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
 201  CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
 251  GGCTAGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
 301  GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
 351  GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
 401  CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
 451  CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
 501  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
 551  TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 601  GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
 651  CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
 701  GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
 751  GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
 801  ACAGGTGTAC ACCCTGCCCC CATGCCGGGA GGAGATGACC AAGAACCAGG
 851  TCAGCCTGTG GTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
 951  CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCGCTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
1101  TAAA                    (SEQ ID NO: 790)
```

The mature ActRIIB-Fc fusion polypeptide is as follows (SEQ ID NO: 800) and may optionally be provided with lysine removed from the C-terminus.

```
   1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
  51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
```

```
101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC
251  REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN RYTQKSLSLS PGK
     (SEQ ID NO: 800)
```

This ActRIIB-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 810):

```
   1  GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
  51  GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
 101  AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
 151  ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
 201  TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
 251  GCTGTGAAGG CAACTTCTGC AACGAGCGCT TCACTCATTT GCCAGAGGCT
 301  GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACCGGTGG
 351  TGGAACTCAC ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGGGGAC
 401  CGTCAGTCTT CCTCTTCCCC CCAAAACCCA AGGACACCCT CATGATCTCC
 451  CGGACCCCTG AGGTCACATG CGTGGTGGTG GACGTGAGCC ACGAAGACCC
 501  TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG CATAATGCCA
 551  AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
 601  GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG
 651  CAAGGTCTCC AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA
 701  AAGCCAAAGG GCAGCCCCGA GAACCACAGG TGTACACCCT GCCCCCATGC
 751  CGGGAGGAGA TGACCAAGAA CCAGGTCAGC CTGTGGTGCC TGGTCAAAGG
 801  CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG
 851  AGAACAACTA CAAGACCACG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC
 901  TTCCTCTATA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA
 951  CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC CGCTACACGC
1001  AGAAGAGCCT CTCCCTGTCT CCGGGTAAA      (SEQ ID NO: 810)
```

The complementary form of ALK4-Fc fusion polypeptide is SEQ ID NO: 403 (shown above), which contains four amino acid substitutions to guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs: 780 and 800 and may optionally be provided with lysine removed from the C-terminus.

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 820):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCCGGGCC CCGGGGGGTC CAGGCTCTGC
 101  TGTGTGCGTG CACCAGCTGC CTCCAGGCCA ACTACACGTG TGAGACAGAT
 151  GGGGCCTGCA TGGTTTCCAT TTTCAATCTG GATGGGATGG AGCACCATGT
 201  GCGCACCTGC ATCCCCAAAG TGGAGCTGGT CCCTGCCGGG AAGCCCTTCT
 251  ACTGCCTGAG CTCGGAGGAC CTGCGCAACA CCCACTGCTG CTACACTGAC
 301  TACTGCAACA GGATCGACTT GAGGGTGCCC AGTGGTCACC TCAAGGAGCC
 351  TGAGCACCCG TCCATGTGGG GCCCGGTGGA GACCGGTGGT GGAACTCACA
 401  CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
 451  CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
 501  GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
 551  TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
 601  CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
 651  CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA
 701  ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
 751  CAGCCCCGAG AACCACAGGT GTGCACCCTG CCCCCATCCC GGGAGGAGAT
 801  GACCAAGAAC CAGGTCAGCC TGTCCTGCGC CGTCAAAGGC TTCTATCCCA
 851  GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC
 901  AAGACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCGTGAG
 951  CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001  GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
1051  TCCCTGTCTC CGGGTAAA      (SEQ ID NO: 820)
```

The mature ALK4-Fc fusion polypeptide sequence is SEQ ID NO: 404 (shown above) and may optionally be provided with lysine removed from the C-terminus.

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 830):

```
   1  TCCGGGCCCC GGGGGGTCCA GGCTCTGCTG TGTGCGTGCA CCAGCTGCCT
  51  CCAGGCCAAC TACACGTGTG AGACAGATGG GGCCTGCATG GTTTCCATTT
 101  TCAATCTGGA TGGGATGGAG CACCATGTGC GCACCTGCAT CCCCAAAGTG
 151  GAGCTGGTCC CTGCCGGGAA GCCCTTCTAC TGCCTGAGCT CGGAGGACCT
 201  GCGCAACACC CACTGCTGCT ACACTGACTA CTGCAACAGG ATCGACTTGA
```

```
251  GGGTGCCCAG TGGTCACCTC AAGGAGCCTG AGCACCCGTC CATGTGGGGC
301  CCGGTGGAGA CCGGTGGTGG AACTCACACA TGCCCACCGT GCCCAGCACC
351  TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
401  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
451  GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT
501  GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA
551  CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT
601  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT
651  CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
701  GCACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
751  TCCTGCGCCG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA
801  GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG
851  ACTCCGACGG CTCCTTCTTC CTCGTGAGCA AGCTCACCGT GGACAAGAGC
901  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
951  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
     (SEQ ID NO: 830)
```

ActRIIB-Fc and ALK4-Fc proteins of SEQ ID NO: 800 and SEQ ID NO: 404, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ALK4-Fc:ActRIIB-Fc.

Purification of various ALK4-Fc:ActRIIB-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography and epitope-based affinity chromatography (e.g., with an antibody or functionally equivalent ligand directed against an epitope on ALK4 or ActRIIB), and multimodal chromatography (e.g., with resin containing both electrostatic and hydrophobic ligands). The purification could be completed with viral filtration and buffer exchange.

Example 2. Ligand binding profile of ActRIIB-Fc:ALK4-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK4-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK4-Fc heterodimeric complex described above with that of ActRIIB-Fc

and ALK4-Fc homodimeric complexes. The ActRIIB-Fc:ALK4-Fc heterodimer, ActRIIB-Fc homodimer, and ALK4-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand off-rate is a particularly significant parameter to evaluate for ligand traps. Soluble receptor-Fc proteins administered in vivo are in constant competition with native receptors for ligands. When endogenous ligands of the TGFbeta superfamily typically bind to cognate receptors at the cell surface, a multi-step signal transduction process is triggered that is relatively slow on a molecular time scale. Native receptors dissociate from ligand slowly in part because significant time is required to generate an intracellular signal from a ligand binding event. For a soluble receptor-Fc protein to comptete effectively for ligand, the off-rate for its complex with the ligand needs to be similar to, or slower than, the off-rate for a ligand complex with native receptor. Ligand binding is a dynamic process and some fraction of ligands will always be in unbound form, so it is important therapeutically for a dose of receptor-Fc protein to capture target ligand for as long as possible. One way to shift the binding equilibrium in favor of more captured ligand is to increase the concentration (dose level) of inhibitor, however this can generate off-target effects that reduce tolerability and safety. A preferable approach is to use an inhibitor with a slower ligand off-rate (longer capture time) combined with ligand binding selectivity to achieve an effective level of ligand antagonism at a lower concentration of inhibitor.

Ligand binding profile of ActRIIB-Fc:ALK4-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK4-Fc homodimer									
Ligand	ActRIIB-Fc homodimer			ALK4-Fc homodimer			ActRIIB-Fc:ALK4-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.2×10^7	2.3×10^{-4}	19	5.8×10^5	1.2×10^{-2}	20000	1.3×10^7	1.5×10^{-4}	12
Activin B	5.1×10^6	1.0×10^{-4}	20	No binding			7.1×10^6	4.0×10^{-5}	6
BMP6	3.2×10^7	6.8×10^{-3}	190	---			2.0×10^6	5.5×10^{-3}	2700
BMP9	1.4×10^7	1.1×10^{-3}	77	---			Transient*		3400
BMP10	2.3×10^7	2.6×10^{-4}	11	---			5.6×10^7	4.1×10^{-3}	74

GDF3	1.4×10^{6}	2.2×10^{-3}	1500	---			3.4×10^{6}	1.7×10^{-2}	4900
GDF8	8.3×10^{5}	2.3×10^{-4}	280	1.3×10^{5}	1.9×10^{-3}	15000 †	3.9×10^{5}	2.1×10^{-4}	550
GDF11	5.0×10^{7}	1.1×10^{-4}	2	5.0×10^{6}	4.8×10^{-3}	270†	3.8×10^{7}	1.1×10^{-4}	3

* Indeterminate due to transient nature of interaction
† Very low signal
--- Not tested

These comparative binding data demonstrate that the ActRIIB-Fc:ALK4-Fc heterodimer has an altered binding profile/selectivity relative to either the ActRIIB-Fc or ALK4-Fc homodimers. The ActRIIB-Fc:ALK4-Fc heterodimer displays enhanced binding to activin B compared with either homodimer, retains strong binding to activin A, GDF8, and GDF11 as observed with ActRIIB-Fc homodimer, and exhibits substantially reduced binding to BMP9, BMP10, and GDF3. In particular, BMP9 displays low or no observable affinity for the ActRIIB-Fc:ALK4-Fc heterodimer, whereas this ligand binds strongly to ActRIIB-Fc homodimer. Like ActRIIB-Fc homodimer, the heterodimer retains intermediate-level binding to BMP6. See **Figure 6**.

These results therefore demonstrate that the ActRIIB-Fc:ALK4-Fc heterodimer is a more selective antagonist of activin A, activin B, GDF8, and GDF11 compared to a ActRIIB-Fc homodimer. Accordingly, an ActRIIB-Fc:ALK4-Fc heterodimer will be more useful than an ActRIIB-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of one or more of activin A, activin B, activin AC, GDF8, and GDF11 but minimize antagonism of one or more of BMP9, BMP10, and BMP6.

Example 3. Activity profile of ActRIIB-Fc:ALK4-Fc heterodimer in mice compared to ActRIIB-Fc homodimer

Homodimeric and heterodimeric complexes were tested in mice to investigate differences in their activity profiles in vivo. Wild-type C57BL/6 mice were dosed subcutaneously with an ActRIIB-Fc homodimer (10 mg/kg), an ActRIIB-Fc:ALK4-Fc heterodimer (3 or 10 mg/kg), or vehicle (phosphate-buffered saline, PBS) twice per week for 4 weeks beginning at approximately 10 weeks of age (n = 9 mice per group). ALK4-Fc homodimer was not tested in vivo due to its inability to bind ligands with high affinity under

cell-free conditions as determined by surface plasmon resonance. Study endpoints included: body weight; total lean mass and total adipose mass as determined by nuclear magnetic resonance (NMR) at baseline and study completion (4 weeks); total bone mineral density as determined by dual energy x-ray absorptiometry (DEXA) at baseline and 4 weeks; and weights of the gastrocnemius, rectus femoris, and pectoralis muscles determined at 4 weeks.

Activity of ActRIIB-Fc and ALK4-Fc Complexes in Wild-Type Mice				
Endpoint (4 wk)	Vehicle	ActRIIB-Fc homodimer 10 mg/kg	ActRIIB-Fc:ALK4-Fc heterodimer	
			10 mg/kg	3 mg/kg
Change in body weight from baseline	↑ 15%	↑ 38% **	↑ 41% **	↑ 33% **
Change in total lean mass from baseline	↓ 1%	↑ 5% **	↑ 5% **	↑ 5% **
Change in total adipose mass from baseline	↑ 5%	↓ 36% **	↓ 35% **	↓ 35% **
Change in total bone mineral density from baseline	↑ 8%	↑ 14% *	↑ 12% *	↑ 11%
Gastrocnemius weight †	23	36 **	35 **	30 **
Femoris weight †	11.5	17 **	16 **	14 **
Pectoralis weight †	15	23 **	28 **	23 **
*$P < 0.05$ vs. vehicle **$P < 0.01$ vs. vehicle † Combined left and right muscle weights normalized to femur length (mg/mm) to control for body size				

Study results are summarized in the table above. As expected, ActRIIB-Fc homodimer caused marked changes in body composition, many consistent with known effects of GDF8 and activin inhibition. Treatment of wild-type mice with ActRIIB-Fc homodimer more than doubled body weight gain over the course of the study compared to vehicle-treated controls. Accompanying this net weight gain were signficant increases in total lean mass and total bone mineral density, as well as a significant reduction in total adipose mass, compared to vehicle. It should be recognized that normalized (percentage-based) changes in lean and adipose tissues differ in their correspondence to absolute changes because lean mass (typically about 70% of body weight in a mouse) is much larger than adipose mass (typically about 10% of body weight). Individual skeletal muscles examined, including the gastrocnemius, femoris, and pectoralis all increased significantly in weight compared to vehicle controls over the course of treatment with ActRIIB-Fc homodimer.

The ActRIIB-Fc:ALK4-Fc heterodimer produced certain effects strikingly similar to those of the ActRIIB-Fc homodimer despite differential ligand selectivity of the two complexes. As shown in the table above, treatment of mice with the ActRIIB-Fc:ALK4-Fc heterodimer at a dose level of 10 mg/kg matched, nearly matched, or exceeded the effects of ActRIIB-Fc homodimer at the same dose level for all endpoints listed. Effects of the ActRIIB-Fc:ALK4-Fc heterodimer at 3 mg/kg were mildly attenuated for several endpoints compared to 10 mg/kg, thus providing evidence for a dose-effect relationship.

Thus, an ActRIIB-Fc:ALK4-Fc heterodimer exerts beneficial anabolic effects on skeletal muscle and bone, and catabolic effects on adipose tissue, very similar to those of ActRIIB-Fc homodimer. However, unlike ActRIIB homodimer, an ActRIIB-Fc:ALK4-Fc heterodimer exhibits only low-affinity or transient binding to BMP9 and BMP10 and so will not concurrently inhibit processes mediated by those ligands, such as angiogenesis. This novel selectivity will be useful, for example, in treating patients in need of stimulatory effects on muscle and bone, and inhibitory effects on fat, but not in need of altered angiogenesis.

Example 4. Generation of an ActRIIB-Fc:ALK3-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK3-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK3, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK3-Fc, respectively.

Formation of heteromeric ActRIIB-Fc:ALK3-Fc may be guided by approaches similar to those described in Example 1.

In a first approach, the polypeptide sequence of the ActRIIB-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 1 as SEQ ID NOs: 100-102.

The complementary ALK3-Fc fusion protein employs the TPA leader and is as follows:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51 LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
101 CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
151 SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
```

```
301 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF LYSDLTVDKS
351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G  (SEQ ID NO: 115)
```

The leader and linker sequences are underlined. To promote formation of the ActRIIB-Fc:ALK3-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 115 may optionally be provided with a lysine added at the C-terminus.

This ALK3-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 116).

```
   1    ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51    AGTCTTCGTT TCGCCCGGCG CCCAGAATCT GGATAGTATG CTTCATGGCA
 101    CTGGGATGAA ATCAGACTCC GACCAGAAAA AGTCAGAAAA TGGAGTAACC
 151    TTAGCACCAG AGGATACCTT GCCTTTTTTA AAGTGCTATT GCTCAGGGCA
 201    CTGTCCAGAT GATGCTATTA ATAACACATG CATAACTAAT GGACATTGCT
 251    TTGCCATCAT AGAAGAAGAT GACCAGGGAG AAACCACATT AGCTTCAGGG
 301    TGTATGAAAT ATGAAGGATC TGATTTTCAG TGCAAAGATT CTCCAAAAGC
 351    CCAGCTACGC CGGACAATAG AATGTTGTCG GACCAATTTA TGTAACCAGT
 401    ATTTGCAACC CACACTGCCC CCTGTTGTCA TAGGTCCGTT TTTTGATGGC
 451    AGCATTCGAA CCGGTGGTGG AACTCACACA TGCCCACCGT GCCCAGCACC
 501    TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
 551    ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
 601    GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT
 651    GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA
 701    CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT
 751    GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT
 801    CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
 851    ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
 901    ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA
 951    GAGCAATGGG CAGCCGGAGA ACAACTACGA CACCACGCCT CCCGTGCTGG
1001    ACTCCGACGG CTCCTTCTTC CTCTATAGCG ACCTCACCGT GGACAAGAGC
1051    AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
1101    GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGT
       (SEQ ID NO: 116)
```

The mature ALK3-Fc fusion protein sequence is as follows (SEQ ID NO: 117) and may optionally be provided with a lysine added at the C-terminus.

```
   1    GAQNLDSMLH GTGMKSDSDQ KKSENGVTLA PEDTLPFLKC YCSGHCPDDA
```

```
 51      INNTCITNGH CFAIIEEDDQ GETTLASGCM KYEGSDFQCK DSPKAQLRRT
101      IECCRTNLCN QYLQPTLPPV VIGPFFDGSI RTGGGTHTCP PCPAPELLGG
151      PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201      KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251      KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301      ENNYDTTPPV LDSDGSFFLY SDLTVDKSRW QQGNVFSCSV MHEALHNHYT
351      QKSLSLSPG            (SEQ ID NO: 117)
```

The ActRIIB-Fc and ALK3-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 117, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK3-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK3-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 407-408, respectively, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK3-Fc fusion polypeptide (SEQ ID NO: 407) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51  LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
101  CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
151  SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
301  SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS
351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK  (SEQ ID NO: 407)
```

The leader sequence and linker are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK3 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 407 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK3-Fc fusion protein sequence (SEQ ID NO: 408) is as follows and may optionally be provided with the lysine (K) removed from the C-terminus.

```
  1  GAQNLDSMLH GTGMKSDSDQ KKSENGVTLA PEDTLPFLKC YCSGHCPDDA
```

```
 51  INNTCITNGH CFAIIEEDDQ GETTLASGCM KYEGSDFQCK DSPKAQLRRT
101  IECCRTNLCN QYLQPTLPPV VIGPFFDGSI RTGGGTHTCP PCPAPELLGG
151  PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201  KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251  KAKGQPREPQ VCTLPPSREE MTKNQVSLSC AVKGFYPSDI AVEWESNGQP
301  ENNYKTTPPV LDSDGSFFLV SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351  QKSLSLSPGK          (SEQ ID NO: 408)
```

The ActRIIB-Fc and ALK3-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 408, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK3-Fc.

Purification of various ActRIIB-Fc:ALK3-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 5. Ligand binding profile of ActRIIB-Fc:ALK3-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK3-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK3-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK3-Fc homodimeric complexes. The ActRIIB-Fc:ALK3-Fc heterodimer, ActRIIB-Fc homodimer, and ALK3-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of ActRIIB-Fc:ALK3-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK3-Fc homodimer									
Ligand	ActRIIB-Fc homodimer			ALK3-Fc homodimer			ActRIIB-Fc:ALK3-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)

Activin A	1.3 x10^{7}	1.4 x10^{-4}	11	No binding			3.4 x10^{7}	5.0 x10^{-3}	150
Activin B	5.1 x10^{6}	1.0 x10^{-4}	20	No binding			2.8 x10^{6}	5.7 x10^{-4}	200
BMP2	Transient*		> 66000	6.8 x10^{5}	8.9 x10^{-5}	130	8.0 x10^{6}	1.1 x10^{-5}	1
BMP4	---			3.0 x10^{5}	5.3 x10^{-5}	180	2.6 x10^{6}	6.5 x10^{-6}	3
BMP5	2.6 x10^{7}	7.5 x10^{-2}	2900	2.9 x10^{4}	2.0 x10^{-3}	70000	9.0 x10^{5}	5.8 x10^{-4}	640
BMP6	3.5 x10^{7}	6.8 x10^{-3}	190	1.4 x10^{5}	4.9 x10^{-3}	35000	2.0 x10^{7}	2.9 x10^{-4}	15
BMP7	8.8 x10^{6}	1.4 x10^{-2}	1600	1.2 x10^{6}	1.8 x10^{-2}	15000	8.2 x10^{5}	1.5 x10^{-3}	1900
BMP9	3.9 x10^{7}	1.3 x10^{-3}	34	No binding			Transient*		>33000
BMP10	5.9 x10^{7}	2.0 x10^{-4}	4	No binding			3.0 x10^{7}	9.4 x10^{-4}	31
GDF3	1.6 x10^{6}	2.3 x10^{-3}	1400	No binding			1.4 x10^{7}	8.2 x10^{-2}	5900
GDF5	Transient*		> 9600	4.8 x10^{5}	1.1 x10^{-2}	22000	1.2 x10^{7}	8.3 x10^{-4}	70
GDF6	---			3.4 x10^{4}	1.3 x10^{-3}	40000	2.8 x10^{5}	4.5 x10^{-4}	1600
GDF7	Transient*		> 12000	2.2 x10^{5}	2.7 x10^{-2}	12000	7.5 x10^{6}	4.0 x10^{-4}	52
GDF8	8.3 x10^{5}	2.3 x10^{-4}	280	No binding			3.0 x10^{6}	9.2 x10^{-4}	310
GDF11	5.0 x10^{7}	1.1 x10^{-4}	2	No binding			1.6 x10^{7}	1.1 x10^{-3}	66

* Indeterminate due to transient nature of interaction
--- Not tested

These comparative binding data demonstrate that the ActRIIB-Fc:ALK3-Fc heterodimer has an altered binding profile/selectivity relative to either the ActRIIB-Fc homodimer or ALK3-Fc homodimer. The ActRIIB-Fc:ALK3-Fc heterodimer binds BMP2 and BMP4 with exceptionally high affinity and displays greatly enhanced binding to BMP5, BMP6, BMP7, GDF5, GDF6, and GDF7 compared with either homodimer. Compared to ActRIIB homodimer, the ActRIIB-Fc:ALK3-Fc heterodimer displays reduced binding to activin A, activin B, BMP10, GDF8, and GDF11 and also discriminates among these ligands to a greater degree, particularly between activin A and activin B. In addition, the ability of ActRIIB-Fc homodimer to bind BMP9 and GDF3 with high affinity is absent for ActRIIB-Fc:ALK3-Fc heterodimer. See **Figure** 7.

These results therefore demonstrate that the ActRIIB-Fc:ALK3-Fc heterodimer is a selective inhibitor of activin B, the GDF5/GDF6/GDF7 ligand subfamily, and several key BMP ligands excluding most notably BMP9. Accordingly, an ActRIIB-Fc:ALK3-Fc heterodimer will be more useful than either an ActRIIB-Fc homodimer or an ALK3-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of BMP2, BMP4, BMP5, and BMP6 or activin B but minimize antagonism of one or more ligands with anabolic muscle effects (e.g., activin A and GDF8) or ligands with angiogenic effects (e.g., BMP9 and BMP10).

Example 6. Activity profile of ActRIIB-Fc:ALK3-Fc heterodimer in mice compared to ActRIIB-Fc homodimer and ALK3-Fc homodimer

Homodimeric and heterodimeric complexes were tested in mice to investigate differences in their activity profiles in vivo. Wild-type C57BL/6 mice were dosed intraperitoneally with ActRIIB-Fc homodimer (10 mg/kg), ALK3-Fc homodimer (10 mg/kg), ActRIIB-Fc:ALK4-Fc heterodimer (3 or 10 mg/kg), or vehicle (phosphate-buffered saline, PBS) twice per week for 6.5 weeks (46 days) beginning at 10 weeks of age (n = 5 mice per group). Study endpoints included body weight, total adipose mass as determined by nuclear magnetic resonance (NMR) at baseline and study completion (6.5 weeks), and total bone mineral density as determined by dual energy x-ray absorptiometry (DEXA) at baseline and 6.5 weeks.

Activity of ActRIIB-Fc and ALK3-Fc Complexes in Wild-Type Mice Compared to Vehicle				
Endpoint 6.5 wk	ActRIIB-Fc homodimer 10 mg/kg	ALK3-Fc homodimer 10 mg/kg	ActRIIB-Fc:ALK3-Fc heterodimer	
			10 mg/kg	3 mg/kg
Body weight	↑ 23% *	↓ 3%	↓ 0.5%	↓ 1%
Total adipose mass	↓ 41% *	↓ 12%	↓ 14% *	↓ 18% *
Total bone mineral density	↑ 8% *	↑ 6% *	↑ 9% *	↑ 10% *
*$P < 0.05$ vs. vehicle				

Study results are summarized in the table above. As expected, the ActRIIB-Fc homodimer significantly increased body weight and total bone mineral density, and

significantly reduced total adipose mass, all compared to vehicle. Also as expected, the ALK3-Fc homodimer significantly increased total bone mineral density compared to vehicle but unlike the ActRIIB-Fc homodimer did not significantly alter either body weight or total adipose mass. The ActRIIB-Fc:ALK3-Fc heterodimer notably displayed an activity profile different from either the ActRIIB-Fc homodimer or the ALK3-Fc homodimer. Treatment of mice with the ActRIIB-Fc:ALK4-Fc heterodimer at either dose level significantly increased bone mineral density at least as well either homodimer. However, unlike ALK3-Fc homodimer, the ActRIIB-Fc:ALK3-Fc heterodimer significantly reduced adipose mass, and unlike ActRIIB-Fc homodimer, the ActRIIB-Fc:ALK3-Fc heterodimer signficantly reduced adipose mass without altering body weight. Thus, an ActRIIB-Fc:ALK3-Fc heterodimer exerts beneficial effects on bone together with potentially beneficial effects on adipose tissue. This novel selectivity will be useful, for example, in treating patients in need of stimulatory effects on bone and inhibitory effects on fat but not in need of altered body weight.

Example 7. Generation of an ActRIIB-Fc:ALK7-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK7-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK7, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK7-Fc, respectively.

Formation of heteromeric ALK7-Fc:ActRIIB-Fc may be guided by approaches similar to those described in Example 1.

In a first approach, the polypeptide sequence of the ActRIIB-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 1 as SEQ ID NOs: 100-102.

The complementary ALK7-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 112):

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251  EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
301  LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
```

(SEQ ID NO: 112)

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK7-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 112 may optionally be provided with a lysine added at the C-terminus.

This ALK7-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 113):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGGACTGAA GTGTGTATGT CTTTTGTGTG
 101  ATTCTTCAAA CTTTACCTGC CAAACAGAAG GAGCATGTTG GGCATCAGTC
 151  ATGCTAACCA ATGGAAAAGA GCAGGTGATC AAATCCTGTG TCTCCCTTCC
 201  AGAACTGAAT GCTCAAGTCT TCTGTCATAG TTCCAACAAT GTTACCAAAA
 251  CCGAATGCTG CTTCACAGAT TTTTGCAACA ACATAACACT GCACCTTCCA
 301  ACAGCATCAC CAAATGCCCC AAAACTTGGA CCCATGGAGA CCGGTGGTGG
 351  AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG GGGGGACCGT
 401  CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG
 451  ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA
 501  GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA
 551  CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC
 601  CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA
 651  GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG
 701  CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG
 751  GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT
 801  CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA
 851  ACAACTACGA CACCACGCCT CCCGTGCTGG ACTCCGACGG CTCCTTCTTC
 901  CTCTATAGCG ACCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT
 951  CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA
1001  AGAGCCTCTC CCTGTCTCCG GGT      (SEQ ID NO: 113)
```

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 114) is expected to be as follows and may optionally be provided with a lysine added at the C-terminus.

```
  1  GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51  CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
201  APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
251  EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ GNVFSCSVMH
301  EALHNHYTQK SLSLSPG        (SEQ ID NO: 114)
```

The ActRIIB-Fc and ALK7-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 114, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK7-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK7-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 405-406, respectively, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK7-Fc fusion polypeptide (SEQ ID NO: 405) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR
251  EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301  LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
       (SEQ ID NO: 405)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK7 fusion polypeptide as indicated by double underline above. Furthermore, the C-terminal lysine residue of the Fc domain can be deleted. The amino acid sequence of SEQ ID NO: 405 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 406) is expected to be as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51  CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
201  APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV KGFYPSDIAV
251  EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH
301  EALHNHYTQK SLSLSPGK        (SEQ ID NO: 406)
```

The ActRIIB-Fc and ALK7-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 406, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK7-Fc.

Purification of various ActRIIB-Fc:ALK7-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 8. Ligand binding profile of ActRIIB-Fc:ALK7-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK7-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK7-Fc homodimeric complexes. The ActRIIB-Fc:ALK7-Fc heterodimer, ActRIIB-Fc homodimer, and ALK7-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of ActRIIB-Fc:ALK7-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer									
Ligand	ActRIIB-Fc homodimer			ALK7-Fc homodimer			ActRIIB-Fc:ALK7-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.3×10^7	1.4×10^{-4}	11	No binding			4.4×10^7	1.9×10^{-3}	43
Activin B	1.5×10^7	1.6×10^{-4}	8	No binding			1.2×10^7	2.0×10^{-4}	17
BMP5	2.6×10^7	7.5×10^{-2}	2900	No binding			1.5×10^5	8.5×10^{-3}	57000
BMP6	2.4×10^7	3.9×10^{-3}	160	No binding			1.2×10^6	6.3×10^{-3}	5300
BMP9	1.2×10^8	1.2×10^{-3}	10	No binding			Transient*		>1400
BMP10	5.9×10^6	1.5×10^{-4}	25	No binding			1.5×10^7	2.8×10^{-3}	190
GDF3	1.4×10^6	2.2×10^{-3}	1500	No binding			2.3×10^6	1.0×10^{-2}	4500
GDF8	3.5×10^6	2.4×10^{-4}	69	No binding			3.7×10^6	1.0×10^{-3}	270
GDF11	9.6×10^7	1.5×10^{-4}	2	No binding			9.5×10^7	7.5×10^{-4}	8
* Indeterminate due to transient nature of interaction									

These comparative binding data demonstrate that the ActRIIB-Fc:ALK7-Fc heterodimer has a different binding profile compared to either the ActRIIB-Fc homodimer or ALK7-Fc homodimer. Interestingly, four of the five ligands with strong binding to ActRIIB-Fc homodimer (activin A, BMP10, GDF8, and GDF11) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer, the exception being activin B which retains tight binding to the heterodimer. In addition, three ligands with intermediate binding to ActRIIB-Fc homodimer (GDF3, BMP6, and particularly BMP9) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer. In contrast, BMP5 binds the ActRIIB-Fc:ALK7 heterodimer with intermediate strength despite only weak binding to ActRIIB-Fc homodimer. No ligands tested bind to ALK7-Fc homodimer. See **Figure 8**.

These results therefore demonstrate that the ActRIIB-Fc:ALK7-Fc heterodimer is a more selective antagonist of activin B in comparison to a ActRIIB-Fc homodimer. Accordingly, an ActRIIB-Fc:ALK7-Fc heterodimer will be more useful than an ActRIIB-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of

activin B but minimize antagonism of one or more of activin A, GDF3, GDF8, GDF11, BMP9, or BMP10.

Example 9. Generation of an ActRIIB-Fc:ALK2-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK2-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK2, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK2-Fc, respectively.

Formation of heteromeric ActRIIB-Fc:ALK2-Fc may be guided by approaches similar to those described in Example 1.

In a first approach, the polypeptide sequence of the ActRIIB-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 1 as SEQ ID NOs: 100-102.

The complementary ALK2-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 136):

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
301  DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPG     (SEQ ID NO: 136)
```

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK2-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 136 may optionally be provided with a lysine added at the C-terminus.

This ALK2-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 137):

```
1    ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
```

```
  51    AGTCTTCGTT TCGCCCGGCG CCATGGAAGA TGAGAAGCCC AAGGTCAACC
 101    CCAAACTCTA CATGTGTGTG TGTGAAGGTC TCTCCTGCGG TAATGAGGAC
 151    CACTGTGAAG GCCAGCAGTG CTTTTCCTCA CTGAGCATCA ACGATGGCTT
 201    CCACGTCTAC CAGAAAGGCT GCTTCCAGGT TTATGAGCAG GGAAAGATGA
 251    CCTGTAAGAC CCCGCCGTCC CCTGGCCAAG CTGTGGAGTG CTGCCAAGGG
 301    GACTGGTGTA ACAGGAACAT CACGGCCCAG CTGCCCACTA AAGGAAAATC
 351    CTTCCCTGGA ACACAGAATT TCCACTTGGA GACCGGTGGT GGAACTCACA
 401    CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
 451    CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
 501    GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
 551    TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
 601    CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
 651    CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA
 701    ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
 751    CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGAGGAGAT
 801    GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA
 851    GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC
 901    GACACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTATAG
 951    CGACCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001    GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
1051    TCCCTGTCTC CGGGT        (SEQ ID NO: 137)
```

The mature ALK2-Fc fusion protein sequence (SEQ ID NO: 138) is as follows and may optionally be provided with a lysine added at the C-terminus.

```
  1  MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
 51  FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
101  HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
251  TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF LYSDLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G   (SEQ ID NO: 138)
```

The ActRIIB-Fc and ALK2-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 138, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK2-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK2-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 421-422, respectively, the Fc domains are altered to

introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK2-Fc fusion polypeptide (SEQ ID NO: 421) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY
301  KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPGK        (SEQ ID NO: 421)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK2 fusion polypeptide as indicated by double underline above. Furthermore, the C-terminal lysine residue of the Fc domain can be deleted. The amino acid sequence of SEQ ID NO: 421 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK2-Fc fusion protein sequence (SEQ ID NO: 422) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
 51  FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
101  HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
251  SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK   (SEQ ID NO: 422)
```

The ActRIIB-Fc and ALK2-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 422, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK2-Fc.

Purification of various ActRIIB-Fc:ALK2-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the

following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 10. Ligand binding profile of ActRIIB-Fc:ALK2-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK2-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK2-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK2-Fc homodimeric complexes. The ActRIIB-Fc:ALK2-Fc heterodimer, ActRIIB-Fc homodimer, and ALK2-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of ActRIIB-Fc:ALK2-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK2-Fc homodimer									
Ligand	ActRIIB-Fc Homodimer			ALK2-Fc Homodimer			ActRIIB-Fc:ALK2-Fc Heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.2×10^7	1.7×10^{-4}	15	No binding			3.4×10^7	2.6×10^{-3}	76
Activin B	3.8×10^6	1.1×10^{-4}	28	No binding			3.2×10^6	1.5×10^{-4}	47
BMP5	3.8×10^6	3.7×10^{-2}	9700	No binding			1.2×10^6	1.4×10^{-3}	1200
BMP7	8.8×10^6	1.4×10^{-2}	1600	No binding			1.5×10^7	2.6×10^{-3}	170
BMP9	3.9×10^7	1.3×10^{-3}	34	No binding			3.2×10^6	8.9×10^{-4}	280
BMP10	5.4×10^7	2.8×10^{-4}	5	No binding			5.5×10^7	2.9×10^{-3}	53
GDF3	1.2×10^6	2.0×10^{-3}	1700	No binding			1.8×10^6	1.2×10^{-2}	6500
GDF5	1.2×10^6	1.4×10^{-3}	1100	No binding			8.8×10^5	4.4×10^{-3}	5000
GDF6	1.5×10^5	5.7×10^{-3}	39000	No binding			Transient*		> 240000
GDF8	2.5×10^6	3.2×10^{-4}	130	No binding			2.1×10^6	7.3×10^{-4}	360

GDF11	2.0 x10^6	2.2 x10^{-4}	110	No binding	1.6 x10^6	9.3 x10^{-4}	600
* Indeterminate due to transient nature of interaction							

These comparative binding data demonstrate that the ActRIIB-Fc:ALK2-Fc heterodimer exhibits a ligand binding profile different from either the ActRIIB-Fc homodimer or the ALK2-Fc homodimer. ActRIIB-Fc:ALK2-Fc heterodimer exhibits preferential and strong binding to activin B, thus resembling ActRIIB-Fc:ALK7-Fc heterodimer (see Example 8). However, ActRIIB-Fc:ALK2-Fc heterodimer differs from ActRIIB-Fc:ALK7-Fc in part by retaining the tight binding to BMP9 characteristic of ActRIIB-Fc homodimer, whereas ActRIIB-Fc:ALK7-Fc binds BMP9 very weakly, if at all. No ligands tested bind to ALK2-Fc homodimer. **See Figure 9**.

These results demonstrate that the ActRIIB-Fc:ALK2-Fc heterodimer is a more selective antagonist of activin B compared to ActRIIB-Fc homodimer. Accordingly, an ActRIIB-Fc:ALK2-Fc heterodimer will be useful in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism primarily of activin B and to supplement that with antagonism secondarily of BMP9, GDF8, and GDF11.

Example 11. Generation of an ActRIIB-Fc:ALK5-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK5-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK5, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK5-Fc, respectively.

Formation of heteromeric ActRIIB-Fc: ALK5-Fc may be guided by approaches similar to those described in Example 1.

In a first approach, the polypeptide sequence of the ActRIIB-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 1 as SEQ ID NOs: 100-102.

The complementary ALK5-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 139):

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
 51  GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
```

```
101  NQDHCNKIEL PTTVKSSPGL GPVETGGGTH TCPPCPAPEL LGGPSVFLFP
151  PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
201  QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
251  EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYDTT
301  PPVLDSDGSF FLYSDLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
351  PG   (SEQ ID NO: 139)
```

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 139 may optionally be provided with a lysine added at the C-terminus.

This ALK5-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 140):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGCGCTGCT CCCGGGGGCG ACGGCGTTAC
 101  AGTGTTTCTG CCACCTCTGT ACAAAAGACA ATTTTACTTG TGTGACAGAT
 151  GGGCTCTGCT TTGTCTCTGT CACAGAGACC ACAGACAAAG TTATACACAA
 201  CAGCATGTGT ATAGCTGAAA TTGACTTAAT TCCTCGAGAT AGGCCGTTTG
 251  TATGTGCACC CTCTTCAAAA ACTGGGTCTG TGACTACAAC ATATTGCTGC
 301  AATCAGGACC ATTGCAATAA AATAGAACTT CCAACTACTG TAAAGTCATC
 351  ACCTGGCCTT GGTCCTGTGG AAACCGGTGG TGGAACTCAC ACATGCCCAC
 401  CGTGCCCAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC
 451  CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG
 501  CGTGGTGGTG GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT
 551  ACGTGGACGG CGTGGAGGTG CATAATGCCA AGACAAAGCC GCGGGAGGAG
 601  CAGTACAACA GCACGTACCG TGTGGTCAGC GTCCTCACCG TCCTGCACCA
 651  GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC AACAAAGCCC
 701  TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
 751  GAACCACAGG TGTACACCCT GCCCCCATCC CGGGAGGAGA TGACCAAGAA
 801  CCAGGTCAGC CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG
 851  CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG AGAACAACTA CGACACCACG
 901  CCTCCCGTGC TGGACTCCGA CGGCTCCTTC TTCCTCTATA GCGACCTCAC
```

```
 951  CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA
1001  TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
1051  CCGGGT   (SEQ ID NO: 140)
```

The mature ALK5-Fc fusion protein sequence (SEQ ID NO: 141) is as follows and may optionally be provided with a lysine added at the C-terminus.

```
   1  ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
  51  DLIPRDRPFV CAPSSKTGSV TTTYCCNQDH CNKIELPTTV KSSPGLGPVE
 101  TGGGTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
 151  EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
 201  YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL
 251  VKGFYPSDIA VEWESNGQPE NNYDTTPPVL DSDGSFFLYS DLTVDKSRWQ
 301  QGNVFSCSVM HEALHNHYTQ KSLSLSPG   (SEQ ID NO: 141)
```

The ActRIIB-Fc and ALK5-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 141, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK5-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK5-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 423-424, respectively, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK5-Fc fusion polypeptide (SEQ ID NO: 423) is as follows:

```
   1  MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
  51  GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
 101  NQDHCNKIEL PTTVKSSPGL GPVETGGGTH TCPPCPAPEL LGGPSVFLFP
 151  PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
 201  QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 251  EPQVCTLPPS REEMTKNQVS LSCAVKGFYP SDIAVEWESN GQPENNYKTT
 301  PPVLDSDGSF FLVSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
 351  PGK      (SEQ ID NO: 423)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four

amino acid substitutions can be introduced into the Fc domain of the ALK5 fusion polypeptide as indicated by double underline above. Furthermore, the C-terminal lysine residue of the Fc domain can be deleted. The amino acid sequence of SEQ ID NO: 423 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK5-Fc fusion protein sequence (SEQ ID NO: 424) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
 51  DLIPRDRPFV CAPSSKTGSV TTTYCCNQDH CNKIELPTTV KSSPGLGPVE
101  TGGGTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
151  EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
201  YKCKVSNKAL PAPIEKTISK AKGQPREPQV CTLPPSREEM TKNQVSLSCA
251  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLVS KLTVDKSRWQ
301  QGNVFSCSVM HEALHNHYTQ KSLSLSPGK      (SEQ ID NO: 424)
```

The ActRIIB-Fc and ALK5-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 424, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK5-Fc.

Purification of various ActRIIB-Fc:ALK5-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 12. Ligand binding profile of ActRIIB-Fc:ALK5-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK5-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK5-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK5-Fc homodimeric complexes. The ActRIIB-Fc:ALK5-Fc heterodimer, ActRIIB-Fc homodimer, and ALK5-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of ActRIIB-Fc:ALK5-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK5-Fc homodimer									
Ligand	ActRIIB-Fc Homodimer			ALK5-Fc Homodimer			ActRIIB-Fc:ALK5-Fc Heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.2×10^7	2.3×10^{-4}	19	No binding			3.6×10^7	1.6×10^{-3}	46
Activin B	5.1×10^6	1.0×10^{-4}	20	No binding			3.9×10^6	3.1×10^{-4}	79
BMP6	6.4×10^6	7.0×10^{-3}	1100	No binding			9.3×10^6	1.5×10^{-2}	1700
BMP9	3.9×10^7	1.3×10^{-3}	34	No binding			Transient*		> 6600
BMP10	2.1×10^7	3.8×10^{-4}	18	No binding			2.3×10^7	2.2×10^{-3}	150
GDF3	4.7×10^5	1.8×10^{-3}	3900	No binding			1.1×10^5	9.7×10^{-3}	8500
GDF8	1.2×10^6	1.9×10^{-4}	160	No binding			1.1×10^6	5.2×10^{-4}	490
GDF11	1.9×10^6	1.4×10^{-4}	74	No binding			2.3×10^6	4.6×10^{-4}	600
* Indeterminate due to transient nature of interaction									

Example 13. Generation of an ActRIIB-Fc:ALK6-Fc heterodimer

A soluble ActRIIB-Fc:ALK6-Fc heteromeric complex can be generated comprising the extracellular domains of human ActRIIB and human ALK6, which can each be fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK6-Fc, respectively.

Formation of heteromeric ActRIIB-Fc: ALK6-Fc may be guided by approaches similar to those described in Example 1.

In a first approach, the polypeptide sequence of the ActRIIB-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 1 as SEQ ID NOs: 100-102.

The complementary ALK6-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 142):

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51  EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLEGSDF QCRDTPIPHQ
101  RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
```

```
151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251  IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW
301  ESNGQPENNY DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA
351  LHNHYTQKSL SLSPG     (SEQ ID NO: 142)
```

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK6-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 142 may optionally be provided with a lysine added at the C-terminus.

This ALK6-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 143):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCAAGAAAGA GGATGGTGAG AGTACAGCCC
 101  CCACCCCCCG TCCAAAGGTC TTGCGTTGTA AATGCCACCA CCATTGTCCA
 151  GAAGACTCAG TCAACAATAT TTGCAGCACA GACGGATATT GTTTCACGAT
 201  GATAGAAGAG GATGACTCTG GGTTGCCTGT GGTCACTTCT GGTTGCCTAG
 251  GACTAGAAGG CTCAGATTTT CAGTGTCGGG ACACTCCCAT TCCTCATCAA
 301  AGAAGATCAA TTGAATGCTG CACAGAAAGG AACGAATGTA ATAAAGACCT
 351  ACACCCTACA CTGCCTCCAT TGAAAAACAG AGATTTTGTT GATGGACCTA
 401  TACACCACAG GACCGGTGGT GGAACTCACA CATGCCCACC GTGCCCAGCA
 451  CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA
 501  GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG
 551  ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC
 601  GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG
 651  CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA
 701  ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC
 751  ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT
 801  GTACACCCTG CCCCCATCCC GGGAGGAGAT GACCAAGAAC CAGGTCAGCC
 851  TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
 901  GAGAGCAATG GGCAGCCGGA GAACAACTAC GACACCACGC CTCCCGTGCT
 951  GGACTCCGAC GGCTCCTTCT TCCTCTATAG CGACCTCACC GTGGACAAGA
```

1001 GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT
1051 CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGT
(SEQ ID NO: 143)

The mature ALK6-Fc fusion protein sequence (SEQ ID NO: 144) is as follows and may optionally be provided with a lysine added at the C-terminus.

1 KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
51 LPVVTSGCLG LEGSDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101 KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251 EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
301 LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
(SEQ ID NO: 144)

The ActRIIB-Fc and ALK6-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 144, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK6-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK6-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 425-426, respectively, the Fc domains can be altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK6-Fc fusion polypeptide (SEQ ID NO: 425) is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
51 EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLEGSDF QCRDTPIPHQ
101 RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151 PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201 VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251 IEKTISKAKG QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW
301 ESNGQPENNY KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA
351 LHNHYTQKSL SLSPGK (SEQ ID NO: 425)

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK6 fusion polypeptide as indicated by double underline above. Furthermore, the C-terminal lysine residue of the Fc domain can be deleted. The amino acid sequence of SEQ ID NO: 425 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK6-Fc fusion protein sequence (SEQ ID NO: 426) can be as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
 51  LPVVTSGCLG LEGSDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101  KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR
251  EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301  LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
     (SEQ ID NO: 426)
```

The ActRIIB-Fc and ALK6-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 426, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK6-Fc.

Purification of various ActRIIB-Fc:ALK6-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 14. Generation of an ActRIIA-Fc:ALK4-Fc heterodimer

Applicants constructed a soluble ActRIIA-Fc:ALK4-Fc heteromeric complex comprising the extracellular domains of human ActRIIA and human ALK4, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIA-Fc fusion polypeptide and ALK4-Fc fusion polypeptide, respectively.

Formation of heteromeric ActRIIA-Fc:ALK4-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face.

The ActRIIA-Fc polypeptide sequence (SEQ ID NO: 118) is shown below:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51 GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101 DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151 CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201 VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251 PAPIEKTISK AKGQPREPQV YTLPPSRKEM TKNQVSLTCL VKGFYPSDIA
301 VEWESNGQPE NNYKTTPPVL KSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
351 HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 118)
```

The leader sequence and linker sequence are underlined. To promote formation of the ActRIIA-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the ActRIIA fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 118 may optionally be provided with the lysine removed from the C-terminus.

This ActRIIA-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 119):

```
  1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51 AGTCTTCGTT TCGCCCGGCG CCGCTATACT TGGTAGATCA GAAACTCAGG
101 AGTGTCTTTT CTTTAATGCT AATTGGGAAA AAGACAGAAC CAATCAAACT
151 GGTGTTGAAC CGTGTTATGG TGACAAAGAT AAACGGCGGC ATTGTTTTGC
201 TACCTGGAAG AATATTTCTG GTTCCATTGA AATAGTGAAA CAAGGTTGTT
251 GGCTGGATGA TATCAACTGC TATGACAGGA CTGATTGTGT AGAAAAAAAA
301 GACAGCCCTG AAGTATATTT CTGTTGCTGT GAGGGCAATA TGTGTAATGA
351 AAAGTTTTCT TATTTTCCGG AGATGGAAGT CACACAGCCC ACTTCAAATC
401 CAGTTACACC TAAGCCACCC ACCGGTGGTG GAACTCACAC ATGCCCACCG
451 TGCCCAGCAC CTGAACTCCT GGGGGGACCG TCAGTCTTCC TCTTCCCCCC
501 AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACATGCG
```

```
 551  TGGTGGTGGA CGTGAGCCAC GAAGACCCTG AGGTCAAGTT CAACTGGTAC
 601  GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCGC GGGAGGAGCA
 651  GTACAACAGC ACGTACCGTG TGGTCAGCGT CCTCACCGTC CTGCACCAGG
 701  ACTGGCTGAA TGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGCCCTC
 751  CCAGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAAAGGGC AGCCCCGAGA
 801  ACCACAGGTG TACACCCTGC CCCCATCCCG GAAGGAGATG ACCAAGAACC
 851  AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTATCCCAG CGACATCGCC
 901  GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACTACA AGACCACGCC
 951  TCCCGTGCTG AAGTCCGACG GCTCCTTCTT CCTCTATAGC AAGCTCACCG
1001  TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
1051  CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
1101  GGGTAAA                    (SEQ ID NO: 119)
```

The mature ActRIIA-Fc fusion polypeptide (SEQ ID NO: 120) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51  IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101  EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151  SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201  SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
251  SRKEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLKSDGS
301  FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
     (SEQ ID NO: 120)
```

In this first approach, the polypeptide sequence of the complementary ALK4-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 1 as SEQ ID NOs: 104-106.

The ActRIIA-Fc and ALK4-Fc proteins of SEQ ID NO: 120 and SEQ ID NO: 106, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIA-Fc:ALK4-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond.

The ActRIIA-Fc polypeptide sequence (SEQ ID NO: 409) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51  GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101  DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151  CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201  VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251  PAPIEKTISK AKGQPREPQV YTLPPCREEM TKNQVSLWCL VKGFYPSDIA
301  VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
351  HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 409)
```

The leader sequence and linker sequence are underlined. To promote formation of the ActRIIA-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 409 may optionally be provided with the lysine removed from the C-terminus.

The mature ActRIIA-Fc fusion polypeptide (SEQ ID NO: 410) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51  IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101  EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151  SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201  SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
251  CREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
301  FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
     (SEQ ID NO: 410)
```

In this second approach, the polypeptide sequence of the complementary ALK4-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 1 as SEQ ID NOs: 403-404.

The ActRIIA-Fc and ALK4-Fc proteins of SEQ ID NO: 410 and SEQ ID NO: 404, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIA-Fc:ALK4-Fc.

Purification of various ActRIIA-Fc:ALK4-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the

following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 15. Ligand binding profile of ActRIIA-Fc:ALK4-Fc heterodimer compared to ActRIIA-Fc homodimer and ALK4-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIA-Fc:ALK4-Fc heterodimeric complex described above with that of ActRIIA-Fc and ALK4-Fc homodimeric complexes. The ActRIIA-Fc:ALK4-Fc heterodimer, ActRIIA-Fc homodimer, and ALK4-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of ActRIIA-Fc:ALK4-Fc heterodimer compared to ActRIIA-Fc homodimer and ALK4-Fc homodimer									
Ligand	ActRIIA-Fc homodimer			ALK4-Fc homodimer			ActRIIA-Fc:ALK4-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.4×10^7	6.2×10^{-4}	45	5.8×10^5	1.2×10^{-2}	20000	7.4×10^6	2.4×10^{-4}	32
Activin B	1.1×10^7	1.1×10^{-4}	10	No binding			9.5×10^6	4.8×10^{-4}	50
Activin AB	2.8×10^7	2.6×10^{-4}	9	1.8×10^6	3.6×10^{-3}	2000	1.8×10^7	2.3×10^{-4}	13
Activin AC	2.2×10^7	7.9×10^{-3}	360	No binding			3.2×10^6	5.4×10^{-4}	170
BMP6	2.7×10^8	2.2×10^{-2}	800	No binding			5.4×10^6	1.2×10^{-2}	2200
BMP7	8.9×10^6	3.3×10^{-2}	3700	No binding			2.0×10^7	7.2×10^{-2}	3500
BMP9	Transient*		>10000	---			No binding		
BMP10	2.9×10^7	2.5×10^{-3}	85	No binding			Transient*		>6000
GDF3	1.5×10^6	3.6×10^{-3}	2400	---			4.9×10^7	4.8×10^{-3}	9800
GDF8	1.4×10^6	1.4×10^{-3}	99	1.3×10^5	1.9×10^{-3}	15000 †	1.8×10^7	2.8×10^{-3}	150

GDF11	7.3 x10^7	9.2 x10^{-4}	13	5.0 x10^6	4.8 x10^{-3}	970†	3.0 x10^7	6.5 x10^{-4}	22
* Indeterminate due to transient nature of interaction † Very low signal --- Not tested									

These comparative binding data demonstrate that the ActRIIA-Fc:ALK4-Fc heterodimer has an altered binding profile/selectivity relative to either the ActRIIA-Fc or ALK4-Fc homodimers. For example, the ActRIIA-Fc:ALK4-Fc heterodimer exhibits enhanced binding to activin A, and particularly enhanced binding to activin AC, compared to ActRIIA-Fc homodimer, while retaining strong binding to activin AB and GDF11. In addition, the ligand with highest affinity for ActRIIA-Fc homodimer, activin B, displays reduced affinity (albeit still within the high-affinity range) for the ActRIIA-Fc:ALK4-Fc heterodimer. The ActRIIA-Fc:ALK4-Fc heterodimer also exhibits markedly reduced binding to BMP10 compared to ActRIIA-Fc homodimer. See **Figure 10**.

These results demonstrate that the ActRIIA-Fc:ALK4-Fc heterodimer is a more selective antagonist of activin A and activin AB over activin B than is ActRIIA-Fc homodimer. In addition, the ActRIIA-Fc:ALK4-Fc heterodimer has substantially increased affinity for activin AC and greatly reduced affinity for BMP10 compared to ActRIIA-Fc homodimer. Accordingly, an ActRIIA-Fc:ALK4-Fc heterodimer will be more useful than ActRIIA-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to antagonize activin A and/or activin AB preferentially over activin B, and to obtain strong inhibition of activin AC, while avoiding inhibition of BMP10.

Example 16. Generation of a BMPRII-Fc:ALK1-Fc heterodimer

Applicants constructed a soluble BMPRII-Fc:ALK1-Fc heteromeric complex comprising the extracellular domains of human BMPRII and human ALK1, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as BMPRII-Fc fusion polypeptide and ALK1-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

Formation of heteromeric BMPRII-Fc:ALK1-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to

introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face.

The BMPRII-Fc polypeptide sequence (SEQ ID NO: 121) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51  HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101  TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151  GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201  EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251  KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG
301  FYPSDIAVEW ESNGQPENNY KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN
351  VFSCSVMHEA LHNHYTQKSL SLSPGK     (SEQ ID NO: 121)
```

The leader sequence and linker sequence are underlined. To promote formation of the BMPRII-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the BMPRII-Fc fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 121 may optionally be provided with the lysine removed from the C-terminus.

This BMPRII-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 122):

```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51  AGTCTTCGTT TCGCCCGGCG CCTCGCAGAA TCAAGAACGC CTATGTGCGT
101  TTAAAGATCC GTATCAGCAA GACCTTGGGA TAGGTGAGAG TAGAATCTCT
151  CATGAAAATG GGACAATATT ATGCTCGAAA GGTAGCACCT GCTATGGCCT
201  TTGGGAGAAA TCAAAAGGGG ACATAAATCT TGTAAAACAA GGATGTTGGT
251  CTCACATTGG AGATCCCCAA GAGTGTCACT ATGAAGAATG TGTAGTAACT
301  ACCACTCCTC CCTCAATTCA GAATGGAACA TACCGTTTCT GCTGTTGTAG
351  CACAGATTTA TGTAATGTCA ACTTTACTGA GAATTTTCCA CCTCCTGACA
401  CAACACCACT CAGTCCACCT CATTCATTTA ACCGAGATGA GACCGGTGGT
451  GGAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC
501  GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC
551  GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT
601  GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA
```

```
 651  GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG
 701  TCCTCACCGT CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC
 751  AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA
 801  AGCCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC
 851  GGAAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
 901  TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA
 951  GAACAACTAC AAGACCACGC CTCCCGTGCT GAAGTCCGAC GGCTCCTTCT
1001  TCCTCTATAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
1051  GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA
1101  GAAGAGCCTC TCCCTGTCTC CGGGTAAA    (SEQ ID NO: 122)
```

The mature BMPRII-Fc fusion polypeptide (SEQ ID NO: 123) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
   1  SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
  51  INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
 101  FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
 151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
 201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
 251  PQVYTLPPSR KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
 301  PVLKSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
 351  GK       (SEQ ID NO: 123)
```

The complementary form of ALK1-Fc fusion polypeptide (SEQ ID NO: 124) is as follows:

```
   1  MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
  51  GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
 101  NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
 151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
 201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
 251  PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP
 301  PVLDSDGSFF LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
 351  G        (SEQ ID NO: 124)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 121 and 123 above, two

amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK1-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 124 may optionally be provided with a lysine added at the C-terminus.

This ALK1-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 125):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGACCCTGT GAAGCCGTCT CGGGGCCCGC
 101  TGGTGACCTG CACGTGTGAG AGCCCACATT GCAAGGGGCC TACCTGCCGG
 151  GGGGCCTGGT GCACAGTAGT GCTGGTGCGG GAGGAGGGGA GGCACCCCCA
 201  GGAACATCGG GGCTGCGGGA ACTTGCACAG GGAGCTCTGC AGGGGCCGCC
 251  CCACCGAGTT CGTCAACCAC TACTGCTGCG ACAGCCACCT CTGCAACCAC
 301  AACGTGTCCC TGGTGCTGGA GGCCACCCAA CCTCCTTCGG AGCAGCCGGG
 351  AACAGATGGC CAGCTGGCCA CCGGTGGTGG AACTCACACA TGCCCACCGT
 401  GCCCAGCACC TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA
 451  AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT
 501  GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG
 551  TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG
 601  TACAACAGCA CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA
 651  CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC
 701  CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA
 751  CCACAGGTGT ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA
 801  GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG
 851  TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACGA CACCACGCCT
 901  CCCGTGCTGG ACTCCGACGG CTCCTTCTTC CTCTATAGCG ACCTCACCGT
 951  GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC
1001  ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
1051  GGT          (SEQ ID NO: 125)
```

The mature ALK1-Fc fusion protein sequence (SEQ ID NO: 126) is as follows and may optionally be provided with a lysine added at the C-terminus.

```
   1  DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
  51  LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
 101  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
```

151 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
201 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV
251 KGFYPSDIAV EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ
301 GNVFSCSVMH EALHNHYTQK SLSLSPG (SEQ ID NO: 126)

The BMPRII-Fc and ALK1-Fc proteins of SEQ ID NO: 123 and SEQ ID NO: 126, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK1-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond.

The BMPRII-Fc polypeptide sequence (SEQ ID NO: 411) is shown below:

1 MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
51 HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101 TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151 GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201 EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251 KVSNKALPAP IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG
301 FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
351 VFSCSVMHEA LHNHYTQKSL SLSPGK (SEQ ID NO: 411)

The leader sequence and linker sequence are underlined. To promote formation of the BMPRII-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 411 may optionally be provided with the lysine removed from the C-terminus.

The mature BMPRII-Fc fusion polypeptide (SEQ ID NO: 412) is as follows and may optionally be provided with the lysine (K) removed from the C-terminus.

1 SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
51 INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
101 FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
151 KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201 YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE

```
251  PQVYTLPPCR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP
301  PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  GK       (SEQ ID NO: 412)
```

The complementary form of ALK1-Fc fusion polypeptide (SEQ ID NO: 413) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
 51  GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
101  NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251  PQVCTLPPSR EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP
301  PVLDSDGSFF LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  GK       (SEQ ID NO: 413)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 411 and 412 above, four amino acid substitutions can be introduced into the Fc domain of the ALK1 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 413 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK1-Fc fusion protein sequence (SEQ ID NO: 414) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
 51  LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
101  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
151  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
201  KCKVSNKALP APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV
251  KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ
301  GNVFSCSVMH EALHNHYTQK SLSLSPGK       (SEQ ID NO: 414)
```

The BMPRII-Fc and ALK1-Fc proteins of SEQ ID NO: 412 and SEQ ID NO: 414, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK1-Fc.

Purification of various BMPRII-Fc:ALK1-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the

following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 17. Ligand binding profile of BMPRII-Fc:ALK1-Fc heterodimer compared to BMPRII-Fc homodimer and ALK1-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the BMPRII-Fc:ALK1-Fc heterodimeric complex described above with that of BMPRII-Fc and ALK1-Fc homodimeric complexes. The BMPRII-Fc:ALK1-Fc heterodimer, BMPRII-Fc homodimer, and ALK1-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of BMPRII-Fc:ALK1-Fc heterodimer compared to BMPRII-Fc homodimer and ALK1-Fc homodimer									
Ligand	BMPRII-Fc homodimer			ALK1-Fc homodimer			BMPRII-Fc:ALK1-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
BMP9	1.2×10^7	2.6×10^{-2}	2100	7.8×10^6	1.3×10^{-4}	16	1.2×10^6	4.1×10^{-4}	360
BMP10	2.6×10^7	2.5×10^{-3}	100	4.1×10^6	1.6×10^{-4}	38	1.5×10^7	3.5×10^{-4}	23
BMP15	9.9×10^6	2.8×10^{-3}	290	No binding			1.2×10^7	4.2×10^{-2}	3500

These comparative binding data demonstrate that the BMPRII-Fc:ALK1-Fc heterodimer has a binding profile/selectivity which differs from that of BMPRII-Fc homodimer but is similar to that of ALK1-Fc homodimer. For example, the BMPRII-Fc:ALK1-Fc heterodimer largely retains the strong binding to BMP9 and BMP10 characteristic of ALK1-Fc homodimer; however, the heterodimer displays modest selectivity for BMP10 over BMP9 not present with the homodimer. Also unlike ALK1-Fc homodimer, the BMPRII-Fc:ALK1-Fc heterodimer binds to BMP15, albeit with an affinity approximately an order of magnitude weaker than that of BMPRII-Fc homodimer. See **Figure 11**. Accordingly, a BMPRII-Fc:ALK1-Fc heterodimer will be unexpectedly useful in certain

therapeutic applications where selective antagonism of BMP9 and particularly BMP10 is advantageous, e.g., for inhibition of angiogenesis, or in applications where antagonism of BMP15 is also advantageous.

Example 18. Generation of a BMPRII-Fc:ALK2-Fc heterodimer

Applicants constructed a soluble BMPRII-Fc:ALK2-Fc heteromeric complex comprising the extracellular domains of human BMPRII and human ALK2, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as BMPRII-Fc fusion polypeptide and ALK2-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric BMPRII-Fc:ALK2-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the BMPRII-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 16 as SEQ ID NOs: 121-123. To promote formation of the BMPRII-Fc:ALK2-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the BMPRII-Fc fusion protein as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 121 and 123 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK2-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 9 as SEQ ID NOs: 136-138. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 121 and 123, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK2-Fc fusion polypeptide as indicated in Example 9. The amino acid sequences of SEQ ID NOs: 136 and 138 may optionally be provided with a lysine added at the C-terminus.

The BMPRII-Fc and ALK2-Fc fusion polypeptides of SEQ ID NO: 123 and SEQ ID NO: 138, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK2-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. BMPRII-Fc fusion polypeptide sequences (SEQ ID NOs: 411-412) are discussed in Example 16. To promote formation of the BMPRII-Fc:ALK2-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the BMPRII-Fc polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 411 and 412 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK2-Fc fusion polypeptide (SEQ ID NOs: 421-422) are discussed in Example 9. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 411-412, four amino acid substitutions can be introduced into the Fc domain of the ALK2 fusion polypeptide as indicated in Example 9. The amino acid sequences of SEQ ID NOs: 421-422 may optionally be provided with the lysine removed from the C-terminus.

The BMPRII-Fc and ALK2-Fc fusion polypeptides of SEQ ID NO: 412 and SEQ ID NO: 422, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK2-Fc.

Purification of various BMPRII-Fc:ALK2-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 19. Ligand binding profile of BMPRII-Fc:ALK2-Fc heterodimer compared to BMPRII-Fc homodimer and ALK2-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the BMPRII-Fc:ALK2-Fc heterodimeric complex described above with that of BMPRII-Fc and ALK2-Fc homodimeric complexes. The BMPRII-Fc:ALK2-Fc heterodimer, BMPRII-Fc homodimer, and ALK2-Fc homodimer were independently captured onto the system using an

anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of BMPRII-Fc:ALK2-Fc heterodimer compared to BMPRII-Fc homodimer and ALK2-Fc homodimer									
Ligand	BMPRII-Fc homodimer			ALK2-Fc homodimer			BMPRII-Fc:ALK2-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin B	1.9×10^6	4.9×10^{-3}	2600	No binding			5.9×10^5	3.1×10^{-3}	5200
BMP5	1.9×10^6	1.9×10^{-2}	9900	No binding			1.8×10^6	5.0×10^{-3}	2800
BMP7	Transient*		> 93000	No binding			1.5×10^7	1.2×10^{-2}	760
BMP9	4.5×10^7	7.3×10^{-2}	1600	No binding			1.0×10^7	5.1×10^{-3}	500
BMP10	3.8×10^7	5.0×10^{-3}	130	No binding			1.1×10^8	3.4×10^{-2}	300
BMP15	5.8×10^6	4.2×10^{-3}	720	No binding			9.6×10^6	1.1×10^{-2}	1100
* Indeterminate due to transient nature of interaction									

Example 20. Generation of a BMPRII-Fc:ALK3-Fc heterodimer

Applicants constructed a soluble BMPRII-Fc:ALK3-Fc heteromeric complex comprising the extracellular domains of human BMPRII and human ALK3, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as BMPRII-Fc fusion polypeptide and ALK3-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric BMPRII-Fc:ALK3-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the BMPRII-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 16 as SEQ ID NOs: 121-123. To promote formation of the BMPRII-Fc:ALK3-Fc

heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the BMPRII-Fc fusion protein as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 121 and 123 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK3-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 4 as SEQ ID NOs: 115-117. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 121 and 123, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK3-Fc fusion polypeptide as indicated in Example 4. The amino acid sequences of SEQ ID NOs: 115 and 117 may optionally be provided with a lysine added at the C-terminus.

The BMPRII-Fc and ALK3-Fc fusion polypeptides of SEQ ID NO: 123 and SEQ ID NO: 117, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK3-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. BMPRII-Fc fusion polypeptide sequences (SEQ ID NOs: 411-412) are discussed in Example 16. To promote formation of the BMPRII-Fc:ALK3-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the BMPRII-Fc polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 411 and 412 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK3-Fc fusion polypeptide (SEQ ID NOs: 407-408) are discussed in Example 4. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 411-412, four amino acid substitutions can be introduced into the Fc domain of the ALK3 fusion polypeptide as indicated in Example 4. The amino acid sequences of SEQ ID NOs: 407 and 408 may optionally be provided with the lysine removed from the C-terminus.

The BMPRII-Fc and ALK3-Fc fusion polypeptides of SEQ ID NO: 412 and SEQ ID NO: 408, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK3-Fc.

Purification of various BMPRII-Fc:ALK3-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 21. Ligand binding profile of BMPRII-Fc:ALK3-Fc heterodimer compared to BMPRII-Fc homodimer and ALK3-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the BMPRII-Fc:ALK3-Fc heterodimeric complex described above with that of BMPRII-Fc and ALK3-Fc homodimeric complexes. The BMPRII-Fc:ALK3-Fc heterodimer, BMPRII-Fc homodimer, and ALK3-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of BMPRII-Fc:ALK3-Fc heterodimer compared to BMPRII-Fc homodimer and ALK3-Fc homodimer									
Ligand	BMPRII-Fc homodimer			ALK3-Fc homodimer			BMPRII-Fc:ALK3-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin B	2.0×10^7	7.5×10^{-2}	3800	No binding			Minimal binding		
BMP2	Transient*		>2 $\times 10^6$	6.2×10^5	1.4×10^{-4}	230	2.9×10^6	1.5×10^{-4}	51
BMP4	---			2.6×10^5	5.5×10^{-5}	210	9.1×10^5	9.1×10^{-5}	100
BMP5	---			2.9×10^4	2.3×10^{-3}	70000	4.3×10^5	1.4×10^{-3}	3200
BMP6	Transient*		>8900	1.4×10^5	4.9×10^{-3}	35000	3.6×10^5	5.9×10^{-4}	1600
BMP7	Transient*		>38000	1.2	1.8	15000	1.2	1.2	1000

				$x10^{6}$	$x10^{-2}$		$x10^{7}$	$x10^{-2}$	
BMP9	$1.2 x10^{7}$	$2.6 x10^{-2}$	2100	No binding			No binding		
BMP10	$2.6 x10^{7}$	$2.5 x10^{-3}$	100	---			$6.8 x10^{5}$	$1.6 x10^{-3}$	2400
BMP15	$9.9 x10^{6}$	$2.8 x10^{-3}$	290	---			$9.1 x10^{5}$	$5.5 x10^{-3}$	6000
GDF5	No binding			$4.3 x10^{5}$	$1.1 x10^{-2}$	22000	Minimal binding		
GDF6	Transient*		>88000	$3.4 x10^{4}$	$1.3 x10^{-3}$	40000	$1.4 x10^{6}$	$1.9 x10^{-3}$	1400
* Indeterminate due to transient nature of interaction --- Not tested									

These comparative binding data demonstrate that the BMPRII-Fc:ALK3-Fc heterodimer has ligand binding selectivity which is clearly unlike that of BMPRII-Fc homodimer but also differs from that of ALK3-Fc homodimer. BMPRII-Fc:ALK3-Fc heterodimer binds much more strongly to BMP6 than does ALK3-Fc homodimer, reflecting an off-rate nearly ten-fold slower. With its largely unchanged binding to BMP2 and BMP4, the BMPRII-Fc:ALK3 heterodimer can therefore be considered a joint inhibitor of BMP2, BMP4, and BMP6. This binding profile contrasts with that of ALK3-Fc homodimer, whose exceptionally strongly binding to BMP4 and BMP2 identifies it as highly selective for this ligand pair compared to four ligands with intermediate-level binding, including BMP6. See **Figure 12**. Accordingly, a BMPRII-Fc:ALK3-Fc heterodimer will be unexpectedly useful in certain therapeutic applications where joint antagonism of BMP2, BMP4, and BMP6 is advantageous.

Example 22. Generation of a BMPRII-Fc:ALK4-Fc heterodimer

Applicants constructed a soluble BMPRII-Fc:ALK4-Fc heteromeric complex comprising the extracellular domains of human BMPRII and human ALK4, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as BMPRII-Fc fusion polypeptide and ALK4-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric BMPRII-Fc:ALK4-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to

introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the BMPRII-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 16 as SEQ ID NOs: 121-123. To promote formation of the BMPRII-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the BMPRII-Fc fusion protein as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 121 and 123 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK4-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 1 as SEQ ID NOs: 104-106. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 121 and 123, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK4-Fc fusion polypeptide as indicated in Example 1. The amino acid sequences of SEQ ID NOs: 104 and 106 may optionally be provided with a lysine added at the C-terminus.

The BMPRII-Fc and ALK4-Fc fusion polypeptides of SEQ ID NO: 123 and SEQ ID NO: 106, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK4-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. BMPRII-Fc fusion polypeptide sequences (SEQ ID NOs: 411 and 412) are discussed in Example 16. To promote formation of the BMPRII-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the BMPRII-Fc polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 411 and 412 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK4-Fc fusion polypeptide (SEQ ID NOs: 403 and 404) are discussed in Example 1. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 411 and 412, four amino acid substitutions can be introduced into the Fc domain of the ALK4 fusion polypeptide as indicated in

Example 1. The amino acid sequences of SEQ ID NOs: 403 and 404 may optionally be provided with the lysine removed from the C-terminus.

The BMPRII-Fc and ALK4-Fc fusion polypeptides of SEQ ID NO: 412 and SEQ ID NO: 404, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK4-Fc.

Purification of various BMPRII-Fc:ALK4-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 23. Ligand binding profile of BMPRII-Fc:ALK4-Fc heterodimer compared to BMPRII-Fc homodimer and ALK4-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the BMPRII-Fc:ALK4-Fc heterodimeric complex described above with that of BMPRII-Fc and ALK4-Fc homodimeric complexes. The BMPRII-Fc:ALK4-Fc heterodimer, BMPRII-Fc homodimer, and ALK4-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of BMPRII-Fc:ALK4-Fc heterodimer compared to BMPRII-Fc homodimer and ALK4-Fc homodimer									
Ligand	BMPRII-Fc homodimer			ALK4-Fc homodimer			BMPRII-Fc:ALK4-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	Transient*		>43000	5.8×10^5	1.2×10^{-2}	20000	2.0×10^6	2.2×10^{-3}	1100
Activin B	2.0×10^7	7.5×10^{-2}	3800	No binding			1.6×10^6	2.6×10^{-3}	1700
Activin AB	---	---	---	4.4×10^6	6.4×10^{-3}	1500	3.6×10^6	5.0×10^{-4}	140
BMP9	1.2×10^7	2.6×10^{-2}	2100	No binding			Transient*		>140000

BMP10	2.6×10^{7}	2.5×10^{-3}	100	No binding	8.0×10^{5}	1.8×10^{-3}	2200
BMP15	9.9×10^{6}	2.8×10^{-3}	290	No binding	2.8×10^{7}	4.8×10^{-2}	1700
* Indeterminate due to transient nature of interaction --- Not tested							

These comparative binding data demonstrate that the BMPRII-Fc:ALK4-Fc heterodimer has ligand binding selectivity which is unlike that of either BMPRII-Fc homodimer or ALK4-Fc homodimer. BMPRII-Fc:ALK4-Fc heterodimer differs from both homodimers by binding several activin ligands with high or intermediate strength and differs from BMPRII-Fc homodimer by binding BMP15 only weakly. Most notably, BMPRII-Fc:ALK4-Fc heterodimer binds strongly and preferentially to the heterodimeric ligand activin AB. See **Figure 13**. Accordingly, a BMPRII-Fc:ALK4-Fc heterodimer will be unexpectedly useful in certain therapeutic applications where antagonism of activin A, activin B, and particularly activin AB are advantageous and where antagonism of BMP15 (which is heavily implicated in ovulation) is to be avoided.

Example 24. Generation of a TGFβRII-Fc:ALK1-Fc heterodimer

Applicants constructed a soluble TGFβRII-Fc:ALK1-Fc heteromeric complex comprising the extracellular domains of the short (canonical) isoform of human TGFβRII and human ALK1, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as $TGF\beta RII_{SHORT}$-Fc fusion polypeptide and ALK1-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric $TGF\beta RII_{SHORT}$-Fc:ALK1-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face.

The $TGF\beta RII_{SHORT}$-Fc polypeptide sequence (SEQ ID NO: 127) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
```

```
151  FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201  EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251  VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRKE
301  MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LKSDGSFFLY
351  SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
     (SEQ ID NO: 127)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{SHORT}$-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the TGFβRII$_{SHORT}$-Fc fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 127 may optionally be provided with the lysine removed from the C-terminus.

This TGFβRII$_{SHORT}$-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 128):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAACTCA
 501  CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT
 551  TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
 601  GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA
 651  GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC
 701  CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC
 751  GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC
 801  CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG
 851  GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGAAGGAG
 901  ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC
```

```
 951  CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT
1001  ACAAGACCAC GCCTCCCGTG CTGAAGTCCG ACGGCTCCTT CTTCCTCTAT
1051  AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC
1101  ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC
1151  TCTCCCTGTC TCCGGGTAAA          (SEQ ID NO: 128)
```

The mature TGFβRII$_{SHORT}$-Fc fusion polypeptide (SEQ ID NO: 129) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
   1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
  51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
 101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GTHTCPPCPA
 151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
 201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
 251  IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW
 301  ESNGQPENNY KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
 351  LHNHYTQKSL SLSPGK      (SEQ ID NO: 129)
```

The polypeptide sequence of the complementary ALK1-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 16 as SEQ ID NOs: 124-126. To guide heterodimer formation with the TGFβRII$_{SHORT}$-Fc fusion polypeptide of SEQ ID NOs: 127 and 129, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK1-Fc fusion polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 124 and 126 may optionally be provided with a lysine added at the C-terminus.

The TGFβRII$_{SHORT}$-Fc and ALK1-Fc proteins of SEQ ID NO: 129 and SEQ ID NO: 126, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{SHORT}$-Fc:ALK1-Fc.

A variant TGFβRII-Fc:ALK1-Fc heteromeric complex may be generated in which the ALK1-Fc polypeptide described above (SEQ ID NO: 126) is paired with an Fc fusion protein comprising the extracellular domain of the long (A) isoform of TGFβRII (TGFβRII$_{LONG}$) in place of the extracellular domain of the short isoform.

The TGFβRII$_{LONG}$-Fc polypeptide sequence (SEQ ID NO: 130) is shown below:

```
   1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
  51  RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
```

101 TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151 KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301 EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ VSLTCLVKGF YPSDIAVEWE
351 SNGQPENNYK TTPPVLKSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
401 HNHYTQKSLS LSPGK (SEQ ID NO: 130)

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{LONG}$-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the TGFβRII$_{LONG}$-Fc fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 130 may optionally be provided with the lysine removed from the C-terminus.

This TGFβRII$_{LONG}$-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 131):

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
101 ATGTGGAAAT GGAGGCCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
151 AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
201 CAACAACGGT GCAGTCAAGT TTCCACAACT GTGTAAATTT TGTGATGTGA
251 GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
301 ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
351 GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCCAAGCTCC
401 CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
451 AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
501 TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
551 ATCCTGACAC CGGTGGTGGA ACTCACACAT GCCCACCGTG CCCAGCACCT
601 GAACTCCTGG GGGGACCGTC AGTCTTCCTC TTCCCCCCAA AACCCAAGGA
651 CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG
701 TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG
751 GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC
801 GTACCGTGTG GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG
851 GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC

```
 901  GAGAAAACCA TCTCCAAAGC CAAAGGGCAG CCCCGAGAAC CACAGGTGTA
 951  CACCCTGCCC CCATCCCGGA AGGAGATGAC CAAGAACCAG GTCAGCCTGA
1001  CCTGCCTGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT GGAGTGGGAG
1051  AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGAA
1101  GTCCGACGGC TCCTTCTTCC TCTATAGCAA GCTCACCGTG GACAAGAGCA
1151  GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG
1201  CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAA
      (SEQ ID NO: 131)
```

The mature TGFβRII$_{LONG}$-Fc fusion polypeptide (SEQ ID NO: 132) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRK
301  EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLKSDGSFFL
351  YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
     (SEQ ID NO: 132)
```

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond.

The TGFβRII$_{SHORT}$-Fc polypeptide sequence (SEQ ID NO: 415) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201  EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251  VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPCREE
301  MTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
351  SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
     (SEQ ID NO: 415)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{SHORT}$-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 415 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{SHORT}$-Fc fusion polypeptide (SEQ ID NO: 416) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GTHTCPPCPA
151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251  IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW
301  ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351  LHNHYTQKSL SLSPGK          (SEQ ID NO: 416)
```

Polypeptide sequences of the complementary ALK1-Fc fusion polypeptide (SEQ ID NOs: 413 and 414) are discussed in Example 16. To guide heterodimer formation with the TGFβRII$_{SHORT}$-Fc fusion polypeptide of SEQ ID NOs: 415 and 416, four amino acid substitutions can be introduced into the Fc domain of the ALK1 fusion polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 413 and 414 may optionally be provided with the lysine removed from the C-terminus.

The TGFβRII$_{SHORT}$-Fc and ALK1-Fc proteins of SEQ ID NO: 416 and SEQ ID NO: 414, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII-Fc:ALK1-Fc.

A variant TGFβRII-Fc:ALK1-Fc heteromeric complex may be generated in which the ALK1-Fc polypeptide described above (SEQ ID NO: 414) is paired with an Fc fusion protein comprising the extracellular domain of the long (A) isoform of TGFβRII (TGFβRII$_{LONG}$) in place of the extracellular domain of the short isoform.

The TGFβRII$_{LONG}$-Fc polypeptide sequence (SEQ ID NO: 417) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51  RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
```

```
101  TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151  KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201  ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251  EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301  EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF YPSDIAVEWE
351  SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
401  HNHYTQKSLS LSPGK   (SEQ ID NO: 417)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{LONG}$-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 417 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{LONG}$-Fc fusion polypeptide (SEQ ID NO: 418) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPCRE
301  EMTKNQVSLW CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
351  YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
     (SEQ ID NO: 418)
```

The TGFβRII$_{LONG}$-Fc and ALK1-Fc proteins of SEQ ID NO: 418 and SEQ ID NO: 414, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{LONG}$-Fc:ALK1-Fc.

Purification of various TGFβRII-Fc:ALK1-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange

chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 25. Ligand binding profile of TGFβRII-Fc:ALK1-Fc heterodimer compared to TGFβRII-Fc homodimer and ALK1-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the TGFβRII$_{SHORT}$-Fc:ALK1-Fc heterodimeric complex described above with that of TGFβRII$_{SHORT}$-Fc and ALK1-Fc homodimeric complexes. The TGFβRII$_{SHORT}$-Fc:ALK1-Fc heterodimer, TGFβRII$_{SHORT}$-Fc homodimer, and ALK1-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of TGFBRII$_{SHORT}$-Fc:ALK1-Fc heterodimer compared to TGFBRII$_{SHORT}$-Fc homodimer and ALK1-Fc homodimer									
Ligand	TGFBRII$_{SHORT}$-Fc homodimer			ALK1-Fc homodimer			TGFBRII$_{SHORT}$-Fc:ALK1-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
BMP9	No binding			7.9×10^6	1.3×10^{-4}	16	2.1×10^7	2.2×10^{-3}	110
BMP10	No binding			1.7×10^7	1.1×10^{-4}	6	1.2×10^7	9.6×10^{-4}	78
TGFβ1	4.2×10^7	1.1×10^{-3}	25	No binding			Transient*		>5300
TGFβ2	Transient*		>44000	No binding			No binding		
TGFβ3	5.9×10^7	5.9×10^{-3}	99	No binding			Transient*		>4700
* Indeterminate due to transient nature of interaction									

Example 26. Generation of a TGFβRII$_{SHORT}$-Fc:ALK5-Fc heterodimer

Applicants constructed a soluble TGFβRII$_{SHORT}$-Fc:ALK5-Fc heteromeric complex comprising the extracellular domains of the human TGFβRII short (canonical) isoform and human ALK5, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred

to as TGFβRII$_{SHORT}$-Fc fusion polypeptide and ALK5-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric TGFβRII$_{SHORT}$-Fc:ALK5-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the TGFβRII$_{SHORT}$-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 24 as SEQ ID NOs: 127-129. To promote formation of the TGFβRII$_{SHORT}$-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the TGFβRII$_{SHORT}$-Fc fusion protein as indicated in Example 24. The amino acid sequences of SEQ ID NOs: 127 and 129 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK5-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 11 as SEQ ID NOs: 139-141. To guide heterodimer formation with the TGFβRII$_{SHORT}$-Fc fusion polypeptide of SEQ ID NOs: 127 and 129, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK5-Fc fusion polypeptide as indicated in Example 11. The amino acid sequences of SEQ ID NOs: 139 and 141 may optionally be provided with a lysine added at the C-terminus.

The TGFβRII$_{SHORT}$-Fc and ALK5-Fc fusion polypeptides of SEQ ID NO: 129 and SEQ ID NO: 141, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{SHORT}$-Fc:ALK5-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. TGFβRII$_{SHORT}$-Fc fusion polypeptide sequences (SEQ ID NOs: 415-416) are discussed in Example 24. To promote formation of the TGFβRII$_{SHORT}$-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the TGFβRII$_{SHORT}$-Fc polypeptide as indicated in Example 24. The amino acid sequences of SEQ ID NOs: 415-416 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK5-Fc fusion polypeptide (SEQ ID NOs: 423-424) are discussed in Example 11. To guide heterodimer formation with the TGFβRII$_{SHORT}$-Fc fusion polypeptide of SEQ ID NOs: 415-416, four amino acid substitutions can be introduced into the Fc domain of the ALK5 fusion polypeptide as indicated in Example 11. The amino acid sequences of SEQ ID NOs: 423-424 may optionally be provided with the lysine removed from the C-terminus.

The TGFβRII$_{SHORT}$-Fc and ALK5-Fc fusion polypeptides of SEQ ID NO: 416 and SEQ ID NO: 424, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{SHORT}$-Fc:ALK5-Fc.

Purification of various TGFβRII$_{SHORT}$-Fc:ALK5-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 27. Generation of a TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer

Applicants constructed a soluble TGFβRII$_{LONG}$-Fc:ALK5-Fc heteromeric complex comprising the extracellular domain of the long (A) isoform of human TGFβRII and the extracellular domain of human ALK5, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as TGFβRII$_{LONG}$-Fc fusion polypeptide and ALK5-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric TGFβRII$_{LONG}$-Fc:ALK5-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the TGFβRII$_{LONG}$-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 24 as SEQ ID NOs: 130-132. To promote formation of the TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the TGFβRII$_{LONG}$-Fc fusion protein as indicated in Example 24. The amino acid

sequences of SEQ ID NOs: 130 and 132 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK5-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 11 as SEQ ID NOs: 139-141. To guide heterodimer formation with the TGFβRII$_{LONG}$-Fc fusion polypeptide of SEQ ID NOs: 130 and 132, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK5-Fc fusion polypeptide as indicated in Example 11. The amino acid sequences of SEQ ID NOs: 139 and 142 may optionally be provided with a lysine added at the C-terminus.

The TGFβRII$_{LONG}$-Fc and ALK5-Fc fusion polypeptides of SEQ ID NO: 132 and SEQ ID NO: 141, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{LONG}$-Fc:ALK5-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. TGFβRII$_{LONG}$-Fc fusion polypeptide sequences (SEQ ID NOs: 417-418) are discussed in Example 24. To promote formation of the TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the TGFβRII$_{LONG}$-Fc polypeptide as indicated in Example 24. The amino acid sequences of SEQ ID NOs: 417-418 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK5-Fc fusion polypeptide (SEQ ID NOs: 423-424) are discussed in Example 11. To guide heterodimer formation with the TGFβRII$_{LONG}$-Fc fusion polypeptide of SEQ ID NOs: 417-418, four amino acid substitutions can be introduced into the Fc domain of the ALK5 fusion polypeptide as indicated in Example 11. The amino acid sequences of SEQ ID NOs: 423-424 may optionally be provided with the lysine removed from the C-terminus.

The TGFβRII$_{LONG}$-Fc and ALK5-Fc fusion polypeptides of SEQ ID NO: 418 and SEQ ID NO: 424, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{LONG}$-Fc:ALK5-Fc.

Purification of various TGFβRII$_{LONG}$-Fc:ALK5-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the

following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 28. Activity profiles of TGFβRII-Fc:ALK5-Fc heterodimers compared to TGFβRII-Fc homodimer and ALK5-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the $TGF\beta RII_{SHORT}$-Fc:ALK5-Fc and $TGF\beta RII_{LONG}$-Fc:ALK5-Fc heterodimeric complexes described in Examples 26-27 with that of $TGF\beta RII_{SHORT}$-Fc and ALK5-Fc homodimeric complexes. The heteromeric or homomeric protein complexes were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profiles of TGFβRII-Fc:ALK5-Fc heterodimers compared to TGFβRII-Fc homodimer and ALK5-Fc homodimer												
Ligand	ALK5-Fc Homodimer			$TGF\beta RII_{SHORT}$-Fc Homodimer			$TGF\beta RII_{SHORT}$-Fc:ALK5-Fc Heterodimer*			$TGF\beta RII_{LONG}$-Fc:ALK5-Fc Heterodimer*		
	k_a	k_d	K_D	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
TGFβ1	No binding			5.6×10^7	1.1×10^{-3}	20	1.4×10^8	1.7×10^{-3}	12	6.6×10^7	9.2×10^{-4}	14
TGFβ2	No binding			2.1×10^5	2.2×10^{-3}	11000	6.6×10^6	2.9×10^{-6}	0.4	4.2×10^6	2.8×10^{-7}	0.07
TGFβ3	No binding			1.9×10^7	1.4×10^{-3}	71	2.7×10^7	1.0×10^{-3}	38	2.7×10^7	1.0×10^{-3}	38
* Low signal which suggests that a substantial fraction of the protein is inactive												

These comparative binding data indicate that the ligand binding profiles of TGFβRII-Fc:ALK5-Fc heterodimers are markedly different from that of TGFβRII-Fc homodimer and from ALK5-Fc homodimer, which did not bind any ligands. Based on the equilibrium dissociation constant (K_D), TGFβRII-Fc homodimer bound TGFβ1 and TGFβ3 with much higher affinity than TGFβ1, even though off-rates for the three TGFβ ligands were similar. In contrast, TGFβRII-Fc:ALK5-Fc heterodimers displayed high selectivity for TGFβ2 over

TGFβ1/TGFβ3. In particular, TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer bound TGFβ2 with an affinity approximately five orders of magnitude higher and an off-rate approximately four orders of magnitude slower than did TGFβRII-Fc homodimer. TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer also bound TGFβ2 more strongly than did heterodimer containing the short isoform. See **Figure 14**. Neither of the TGFβRII-Fc:ALK5-Fc heterodimers was able to bind BMP9 or BMP10 (data not shown), which distinguishes these TGFβRII-Fc:ALK5-Fc heterodimers from TGFβRII-Fc:ALK1-Fc heterodimer (see Example 25). Sensograms for the two TGFβRII-Fc:ALK5-Fc heterodimers exhibited low signal amplitude which suggests that a substantial fraction of each protein was inactive.

To better interpret these data obtained by surface plasmon resonance, a reporter gene assay in A549 cells was used to determine the ability of TGFβRII fusion proteins to inhibit activity of TGFβ1, TGFβ2, and TGFβ3. This assay is based on a human lung carcinoma cell line transfected with reporter plasmids pGL3(CAGA)12-firefly luciferase (Dennler et al, 1998, EMBO 17: 3091-3100) and pRLCMV-renilla luciferase, the latter to control for transfection efficiency. The CAGA motif is present in the promoters of TGFβ-responsive genes (for example, PAI-1), so this vector is of general use for factors signaling through SMAD2 and SMAD3.

On the first day of the assay, A549 cells (ATCC®: CCL-185™) were distributed in 48-well plates at $6.5x10^4$ cells per well and incubated overnight. All incubations were at 37°C and 5% CO_2 in a tissue culture incubator unless otherwise indicated. On the second day, a solution containing 10 µg pGL3(CAGA)12-firefly luciferase, 100 ng pRLCMV-renilla luciferase, 30 µL X-tremeGENE 9 (Roche Applied Science), and 970 µL OptiMEM (Invitrogen) was preincubated for 30 min at room temperature, then added to 24 mL Eagle's minimum essential medium (EMEM, ATCC®) supplemented with 0.1% BSA. Medium was removed from the plated cells and this transfection mixture was applied to the cells (500 µl/well) for an overnight incubation. On the third day, medium was removed, and cells were incubated overnight with a mixture of ligands and inhibitors prepared as described below.

Serial dilutions of test articles were made in a 48-well plate in a 200 µL volume of assay buffer (EMEM + 0.1 % BSA). An equal volume of assay buffer containing the test ligand was added to obtain a final ligand concentration equal to the EC_{50} determined previously. Human TGFβ1, TGFβ2, and TGFβ3 were obtained from PeproTech. Test solutions were incubated for 30 minutes, then 250 µL of the mixture was added to the

transfected cells. Each concentration of test article was determined in duplicate. After incubation with test solutions overnight, cells were rinsed with phosphate-buffered saline, then lysed with passive lysis buffer (Promega E1941) and stored overnight at -70°C. On the fourth and final day, plates were warmed to room temperature with gentle shaking. Cell lysates were transferred to a chemiluminescence plate (96-well) and analyzed in a luminometer with reagents from a Dual-Luciferase Reporter Assay system (Promega E1980) to determine normalized luciferase activity.

This assay was used to compare the ability of TGFβRII fusion protein variants to inhibit cell signaling by TGFβRII ligands. Results are shown in the table below.

Inhibitory Activity of TGFβRII Fusion Proteins in A549 Cells			
Construct	IC_{50} (pM)		
	TGFβ1 (640 pg/mL)	TGFβ2 (480 pg/mL)	TGFβ3 (270 pg/mL)
$TGF\beta RII_{SHORT}$-Fc homodimer	90	---	9
$TGF\beta RII_{SHORT}$-Fc:ALK5-Fc heterodimer*	< 350*	~ 200*	< 90*
$TGF\beta RII_{LONG}$-Fc:ALK5-Fc heterodimer	204	154	35
--- No inhibition (tested at concentrations up to 10 nM) * Value imprecise due to range of concentrations tested			

Results with TGFβRII-Fc homodimer were consistent with previous reports concerning wild-type $TGF\beta RII_{SHORT}$-Fc and $TGF\beta RII_{LONG}$-Fc homodimers (del Re et al., J Biol Chem 279:22765, 2004). In this experiment, $TGF\beta RII_{SHORT}$-Fc homodimer potently inhibited TGFβ1 and TGFβ3 but was unable to inhibit TGFβ2 at homodimer concentrations up to 10 nM. This finding is consistent with the low affinity of TGFβ2 binding to TGFβRII-Fc homodimer but oddly inconsistent with its slow off-rate (see binding results above). In contrast, TGFβRII-Fc:ALK5-Fc heterodimers potently inhibited all three TGFβ ligands in a cellular environment. Accordingly, a TGFβRII-Fc:ALK5-Fc heterodimer will be unexpectedly useful in certain therapeutic applications where preferential antagonism of TGFβ2 – or combined antagonism of TGFβ1, TGFβ2, and TGFβ3 – are advantageous.

```
   1 ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGGAT CGCTGTGCGC
  51 CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
 101 CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA
 151 GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
 201 TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
 251 GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
 301 TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC
 351 AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
 401 CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC
 451 CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA
 501 CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC
 551 TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
 601 TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA
 651 GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT
 701 TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC
 751 GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT
 801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951 TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA
1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101 TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201 AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251 GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA
1301 AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351 GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC
1401 TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC
1501 ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC (SEQ ID NO: 7)
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  1 GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
 51 GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
101 AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
151 ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
201 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
251 GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
301 GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
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(SEQ ID NO: 8)

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   1 ATGGGAGCTG CTGCAAAGTT GGCGTTTGCC GTCTTTCTTA TCTCCTGTTC
  51 TTCAGGTGCT ATACTTGGTA GATCAGAAAC TCAGGAGTGT CTTTTCTTTA
 101 ATGCTAATTG GGAAAAAGAC AGAACCAATC AAACTGGTGT TGAACCGTGT
 151 TATGGTGACA AAGATAAACG GCGGCATTGT TTTGCTACCT GGAAGAATAT
 201 TTCTGGTTCC ATTGAAATAG TGAAACAAGG TTGTTGGCTG GATGATATCA
 251 ACTGCTATGA CAGGACTGAT TGTGTAGAAA AAAAAGACAG CCCTGAAGTA
 301 TATTTTTGTT GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATTT
 351 TCCGGAGATG GAAGTCACAC AGCCCACTTC AAATCCAGTT ACACCTAAGC
 401 CACCCTATTA CAACATCCTG CTCTATTCCT TGGTGCCACT TATGTTAATT
 451 GCGGGGATTG TCATTTGTGC ATTTTGGGTG TACAGGCATC ACAAGATGGC
 501 CTACCCTCCT GTACTTGTTC CAACTCAAGA CCCAGGACCA CCCCCACCTT
 551 CTCCATTACT AGGTTTGAAA CCACTGCAGT TATTAGAAGT GAAAGCAAGG
 601 GGAAGATTTG GTTGTGTCTG GAAAGCCCAG TTGCTTAACG AATATGTGGC
 651 TGTCAAAATA TTTCCAATAC AGGACAAACA GTCATGGCAA AATGAATACG
 701 AAGTCTACAG TTTGCCTGGA ATGAAGCATG AGAACATATT ACAGTTCATT
 751 GGTGCAGAAA AACGAGGCAC CAGTGTTGAT GTGGATCTTT GGCTGATCAC
 801 AGCATTTCAT GAAAAGGGTT CACTATCAGA CTTTCTTAAG GCTAATGTGG
 851 TCTCTTGGAA TGAACTGTGT CATATTGCAG AAACCATGGC TAGAGGATTG
 901 GCATATTTAC ATGAGGATAT ACCTGGCCTA AAAGATGGCC ACAAACCTGC
 951 CATATCTCAC AGGGACATCA AAAGTAAAAA TGTGCTGTTG AAAAACAACC
1001 TGACAGCTTG CATTGCTGAC TTTGGGTTGG CCTTAAAATT TGAGGCTGGC
1051 AAGTCTGCAG GCGATACCCA TGGACAGGTT GGTACCCGGA GGTACATGGC
1101 TCCAGAGGTA TTAGAGGGTG CTATAAACTT CCAAAGGGAT GCATTTTTGA
1151 GGATAGATAT GTATGCCATG GGATTAGTCC TATGGGAACT GGCTTCTCGC
1201 TGTACTGCTG CAGATGGACC TGTAGATGAA TACATGTTGC CATTTGAGGA
1251 GGAAATTGGC CAGCATCCAT CTCTTGAAGA CATGCAGGAA GTTGTTGTGC
1301 ATAAAAAAAA GAGGCCTGTT TTAAGAGATT ATTGGCAGAA ACATGCTGGA
1351 ATGGCAATGC TCTGTGAAAC CATTGAAGAA TGTTGGGATC ACGACGCAGA
1401 AGCCAGGTTA TCAGCTGGAT GTGTAGGTGA AAGAATTACC CAGATGCAGA
1451 GACTAACAAA TATTATTACC ACAGAGGACA TTGTAACAGT GGTCACAATG
1501 GTGACAAATG TTGACTTTCC TCCCAAAGAA TCTAGTCTA
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(SEQ ID NO: 12)

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  1 ATACTTGGTA GATCAGAAAC TCAGGAGTGT CTTTTCTTTA ATGCTAATTG
 51 GGAAAAAGAC AGAACCAATC AAACTGGTGT TGAACCGTGT TATGGTGACA
101 AAGATAAACG GCGGCATTGT TTTGCTACCT GGAAGAATAT TTCTGGTTCC
151 ATTGAAATAG TGAAACAAGG TTGTTGGCTG GATGATATCA ACTGCTATGA
201 CAGGACTGAT TGTGTAGAAA AAAAAGACAG CCCTGAAGTA TATTTTTGTT
251 GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATTT TCCGGAGATG
301 GAAGTCACAC AGCCCACTTC AAATCCAGTT ACACCTAAGC CACCC
```

(SEQ ID NO: 13)

ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGC
CAGCACGATCCCACCGCACGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACA
ACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGAC
AACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGT
CTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACC
CCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAG
GAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGA
CAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTC
AAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGTCATCATCATCTTC
TACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAACCGGCAAGACGCG
GAAGCTCATGGAGTTCAGCGAGCACTGTGCCATCATCCTGGAAGATGACCGCTCTGACATCA
GCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCCATTGAGCTGGACACC
CTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGCAGAACACTTCAGA
GCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCCTCTTGGAAGACAG
AGAAGGACATCTTCTCAGACATCAATCTGAAGCATGAGAACATACTCCAGTTCCTGACGGCT
GAGGAGCGGAAGACGGAGTTGGGGAAACAATACTGGCTGATCACCGCCTTCCACGCCAAGGG
CAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGACCTGCGCAAGCTGGGCA
GCTCCCTCGCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATGTGGGAGGCCCAAG
ATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGAACGACCTAACCTG
CTGCCTGTGTGACTTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCTGTGGATGACCTGG
CTAACAGTGGGCAGGTGGGAACTGCAAGATACATGGCTCCAGAAGTCCTAGAATCCAGGATG
AATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACTCCATGGCTCTGGTGCTCTG
GGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAGCCTCCATTTGGTT
CCAAGGTGCGGGAGCACCCCTGTGTCGAAAGCATGAAGGACAACGTGTTGAGAGATCGAGGG
CGACCAGAAATTCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGGTGTGTGAGACGTT
GACTGAGTGCTGGGACCACGACCCAGAGGCCCGTCTCACAGCCCAGTGTGTGGCAGAACGCT
TCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGCTGCTCGGAGGAGAAGATTCCT
GAAGACGGCTCCCTAAACACTACCAAA (SEQ ID NO: 44)

ACGATCCCACCGCACGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACAACGG
TGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACC
AGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGTCTGT
GTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACCCCAA
GCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAA
AAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGACAAC
ATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAA
(SEQ ID NO: 45)

ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGC
CAGCACGATCCCACCGCACGTTCAGAAGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAA
TCATCTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATA
GTCACTGACAACAACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATT
TTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGA
AGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACA
GTTTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAA
GTGCATTATGAAGGAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTG
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CTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGT
CATCATCATCTTCTACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAA
CCGGCAAGACGCGGAAGCTCATGGAGTTCAGCGAGCACTGTGCCATCATCCTGGAAGATGAC
CGCTCTGACATCAGCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCCAT
TGAGCTGGACACCCTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGC
AGAACACTTCAGAGCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCC
TCTTGGAAGACAGAGAAGGACATCTTCTCAGACATCAATCTGAAGCATGAGAACATACTCCA
GTTCCTGACGGCTGAGGAGCGGAAGACGGAGTTGGGGAAACAATACTGGCTGATCACCGCCT
TCCACGCCAAGGGCAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGACCTG
CGCAAGCTGGGCAGCTCCCTCGCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATG
TGGGAGGCCCAAGATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGA
ACGACCTAACCTGCTGCCTGTGTGACTTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCT
GTGGATGACCTGGCTAACAGTGGGCAGGTGGGAACTGCAAGATACATGGCTCCAGAAGTCCT
AGAATCCAGGATGAATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACTCCATGG
CTCTGGTGCTCTGGGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAG
CCTCCATTTGGTTCCAAGGTGCGGGAGCACCCCTGTGTCGAAAGCATGAAGGACAACGTGTT
GAGAGATCGAGGGCGACCAGAAATTCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGG
TGTGTGAGACGTTGACTGAGTGCTGGGACCACGACCCAGAGGCCCGTCTCACAGCCCAGTGT
GTGGCAGAACGCTTCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGCTGCTCGGA
GGAGAAGATTCCTGAAGACGGCTCCCTAAACACTACCAAA (SEQ ID NO: 69)

ACGATCCCACCGCACGTTCAGAAGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAATCAT
CTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATAGTCA
CTGACAACAACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCC
ACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCC
ACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTT
GCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGC
ATTATGAAGGAAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGA
GTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAG
TCATATTTCAA (SEQ ID NO: 70).

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGT
CAGCACTGCGGCTGCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGC
AAGACCTTGGGATAGGTGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAA
GGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGG
ATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCA
CTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAAT
GTCAACTTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATT
TAACCGAGATGAGACAATAATCATTGCTTTGGCATCAGTCTCTGTATTAGCTGTTTTGATAG
TTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAAACAAGGTCTTCACAGTATG
AACATGATGGAGGCAGCAGCATCCGAACCCTCTCTTGATCTAGATAATCTGAAACTGTTGGA
GCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTG
CTGTAAAAGTGTTTTCCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGA
GTGCCTTTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGC
AGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATCCCAATGGATCTTTATGCAAGT
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GGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCA
TCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACT
TTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCA
GCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAA
CTTGAGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGACTAATCTATT
GGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATG
GCTTTTCAGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAGGTTCTCGTGTCTAG
GGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCAC
TCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGT
GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGCAACCTGTCACATAATAGGCGTGTGC
CAAAAATTGGTCCTTATCCAGATTATTCTTCCTCCTCATACATTGAAGACTCTATCCATCAT
ACTGACAGCATCGTGAAGAATATTTCCTCTGAGCATTCTATGTCCAGCACACCTTTGACTAT
AGGGGAAAAAAACCGAAATTCAATTAACTATGAACGACAGCAAGCACAAGCTCGAATCCCCA
GCCCTGAAACAAGTGTCACCAGCCTCTCCACCAACACAACAACCACAAACACCACAGGACTC
ACGCCAAGTACTGGCATGACTACTATATCTGAGATGCCATACCCAGATGAAACAAATCTGCA
TACCACAAATGTTGCACAGTCAATTGGGCCAACCCCTGTCTGCTTACAGCTGACAGAAGAAG
ACTTGGAAACCAACAAGCTAGACCCAAAAGAAGTTGATAAGAACCTCAAGGAAAGCTCTGAT
GAGAATCTCATGGAGCACTCTCTTAAACAGTTCAGTGGCCCAGACCCACTGAGCAGTACTAG

TTCTAGCTTGCTTTACCCACTCATAAAACTTGCAGTAGAAGCAACTGGACAGCAGGACTTCA
CACAGACTGCAAATGGCCAAGCATGTTTGATTCCTGATGTTCTGCCTACTCAGATCTATCCT
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AACAAAAGAGCCCCGGCTAAAATTTGGCAGCAAGCACAAATCAAACTTGAAACAAGTCGAAA
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TGGGACAGTACTATCTGGCCAAACAACCAACATAGTGACACATAGGGCCCAAGAAATGTTGC
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CCTTTACTGAGACGAGAGCAACAAGCTGGCCATGATGAAGGTGTTCTGGATCGTCTTGTGGA
CAGGAGGGAACGGCCACTAGAAGGTGGCCGAACTAATTCCAATAACAACAACAGCAATCCAT
GTTCAGAACAAGATGTTCTTGCACAGGGTGTTCCAAGCACAGCAGCAGATCCTGGGCCATCA
AAGCCCAGAAGAGCACAGAGGCCTAATTCTCTGGATCTTTCAGCCACAAATGTCCTGGATGG
CAGCAGTATACAGATAGGTGAGTCAACACAAGATGGCAAATCAGGATCAGGTGAAAAGATCA
AGAAACGTGTGAAAACTCCCTATTCTCTTAAGCGGTGGCGCCCCTCCACCTGGGTCATCTCC
ACTGAATCGCTGGACTGTGAAGTCAACAATAATGGCAGTAACAGGGCAGTTCATTCCAAATC
CAGCACTGCTGTTTACCTTGCAGAAGGAGGCACTGCTACAACCATGGTGTCTAAAGATATAG
GAATGAACTGTCTG (SEQ ID NO: 48)

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGG
TGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATG
GCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGGATGTTGGTCTCACATT
GGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCACTCCTCCCTCAATTCA
GAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGAGA
ATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
(SEQ ID NO: 49)

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGT
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AAGACCTTGGGATAGGTGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAA
GGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGG
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GTGCCTTTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGC
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GGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCA
TCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACT
TTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCA
GCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAA
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GGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATG
GCTTTTCAGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAGGTTCTCGTGTCTAG
GGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCAC
TCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGT
GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGTAGG (SEQ ID NO: 73)

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGG
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GCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGGATGTTGGTCTCACATT
GGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCACTCCTCCCTCAATTCA
GAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGAGA
ATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
(SEQ ID NO: 74)

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
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TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
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CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
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CCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCA
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CCTTGTTCCAGGAATCCTCAGCCTGCCTGTACCCTTTCTCCTGTG (SEQ ID NO: 52)

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGAC
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GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 53)

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
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TGATGGGC (SEQ ID NO: 77)

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGAC
ACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCC
GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC
CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
GCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCA
GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 78)

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
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TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
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CAAGGCCCAGCTGCCATCATGGAAGACCCTGATGGGCTGAGGGAGCTCCTAGAAGACTGTTG
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CCCATCCTCAAGAGAGCCACCCCTTTCCAGAGAGCTGTCCACGTGGCTGCCCACCTCTCTGC
CCAGAAGACTGTACTTCAATTCCTGCCCCTACCATCCTCCCCTGTAGGCCTCAGCGGAGTGC
CTGCCACTTCAGCGTTCAGCAAGGCCCTTGTTCCAGGAATCCTCAGCCTGCCTGTACCCTTT
CTCCTGTG (SEQ ID NO: 81)

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGAC
ACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCC
GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC
CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
GCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCA
GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 82)

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   1 ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGGAT CGCTGTGCGC
  51 CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
 101 CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA
 151 GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
 201 TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
 251 GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
 301 TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC
 351 AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
 401 CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC
 451 CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA
 501 CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC
 551 TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
 601 TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA
 651 GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT
 701 TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC
 751 GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT
 801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951 TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA
1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101 TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201 AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251 GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA
1301 AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351 GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC
1401 TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC
1501 ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC (SEQ ID NO: 7)
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  1 GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
 51 GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
101 AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
151 ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
201 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
251 GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
301 GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
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(SEQ ID NO: 8)

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   1 ATGGGAGCTG CTGCAAAGTT GGCGTTTGCC GTCTTTCTTA TCTCCTGTTC
  51 TTCAGGTGCT ATACTTGGTA GATCAGAAAC TCAGGAGTGT CTTTTCTTTA
 101 ATGCTAATTG GGAAAAAGAC AGAACCAATC AAACTGGTGT TGAACCGTGT
 151 TATGGTGACA AAGATAAACG GCGGCATTGT TTTGCTACCT GGAAGAATAT
 201 TTCTGGTTCC ATTGAAATAG TGAAACAAGG TTGTTGGCTG GATGATATCA
 251 ACTGCTATGA CAGGACTGAT TGTGTAGAAA AAAAAGACAG CCCTGAAGTA
 301 TATTTTTGTT GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATTT
 351 TCCGGAGATG GAAGTCACAC AGCCCACTTC AAATCCAGTT ACACCTAAGC
 401 CACCCTATTA CAACATCCTG CTCTATTCCT TGGTGCCACT TATGTTAATT
 451 GCGGGGATTG TCATTTGTGC ATTTTGGGTG TACAGGCATC ACAAGATGGC
 501 CTACCCTCCT GTACTTGTTC CAACTCAAGA CCCAGGACCA CCCCCACCTT
 551 CTCCATTACT AGGTTTGAAA CCACTGCAGT TATTAGAAGT GAAAGCAAGG
 601 GGAAGATTTG GTTGTGTCTG GAAAGCCCAG TTGCTTAACG AATATGTGGC
 651 TGTCAAAATA TTTCCAATAC AGGACAAACA GTCATGGCAA AATGAATACG
 701 AAGTCTACAG TTTGCCTGGA ATGAAGCATG AGAACATATT ACAGTTCATT
 751 GGTGCAGAAA AACGAGGCAC CAGTGTTGAT GTGGATCTTT GGCTGATCAC
 801 AGCATTTCAT GAAAAGGGTT CACTATCAGA CTTTCTTAAG GCTAATGTGG
 851 TCTCTTGGAA TGAACTGTGT CATATTGCAG AAACCATGGC TAGAGGATTG
 901 GCATATTTAC ATGAGGATAT ACCTGGCCTA AAAGATGGCC ACAAACCTGC
 951 CATATCTCAC AGGGACATCA AAAGTAAAAA TGTGCTGTTG AAAAACAACC
1001 TGACAGCTTG CATTGCTGAC TTTGGGTTGG CCTTAAAATT TGAGGCTGGC
1051 AAGTCTGCAG GCGATACCCA TGGACAGGTT GGTACCCGGA GGTACATGGC
1101 TCCAGAGGTA TTAGAGGGTG CTATAAACTT CCAAAGGGAT GCATTTTTGA
1151 GGATAGATAT GTATGCCATG GGATTAGTCC TATGGGAACT GGCTTCTCGC
1201 TGTACTGCTG CAGATGGACC TGTAGATGAA TACATGTTGC CATTTGAGGA
1251 GGAAATTGGC CAGCATCCAT CTCTTGAAGA CATGCAGGAA GTTGTTGTGC
1301 ATAAAAAAAA GAGGCCTGTT TTAAGAGATT ATTGGCAGAA ACATGCTGGA
1351 ATGGCAATGC TCTGTGAAAC CATTGAAGAA TGTTGGGATC ACGACGCAGA
1401 AGCCAGGTTA TCAGCTGGAT GTGTAGGTGA AAGAATTACC CAGATGCAGA
1451 GACTAACAAA TATTATTACC ACAGAGGACA TTGTAACAGT GGTCACAATG
1501 GTGACAAATG TTGACTTTCC TCCCAAAGAA TCTAGTCTA
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(SEQ ID NO: 12)

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  1 ATACTTGGTA GATCAGAAAC TCAGGAGTGT CTTTTCTTTA ATGCTAATTG
 51 GGAAAAAGAC AGAACCAATC AAACTGGTGT TGAACCGTGT TATGGTGACA
101 AAGATAAACG GCGGCATTGT TTTGCTACCT GGAAGAATAT TTCTGGTTCC
151 ATTGAAATAG TGAAACAAGG TTGTTGGCTG GATGATATCA ACTGCTATGA
201 CAGGACTGAT TGTGTAGAAA AAAAAGACAG CCCTGAAGTA TATTTTTGTT
251 GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATTT TCCGGAGATG
301 GAAGTCACAC AGCCCACTTC AAATCCAGTT ACACCTAAGC CACCC
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(SEQ ID NO: 13)

ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGC
CAGCACGATCCCACCGCACGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACA
ACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGAC
AACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGT
CTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACC
CCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAG
GAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGA
CAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTC
AAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGTCATCATCATCTTC
TACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAACCGGCAAGACGCG
GAAGCTCATGGAGTTCAGCGAGCACTGTGCCATCATCCTGGAAGATGACCGCTCTGACATCA
GCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCCATTGAGCTGGACACC
CTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGCAGAACACTTCAGA
GCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCCTCTTGGAAGACAG
AGAAGGACATCTTCTCAGACATCAATCTGAAGCATGAGAACATACTCCAGTTCCTGACGGCT
GAGGAGCGGAAGACGGAGTTGGGGAAACAATACTGGCTGATCACCGCCTTCCACGCCAAGGG
CAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGACCTGCGCAAGCTGGGCA
GCTCCCTCGCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATGTGGGAGGCCCAAG
ATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGAACGACCTAACCTG
CTGCCTGTGTGACTTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCTGTGGATGACCTGG
CTAACAGTGGGCAGGTGGGAACTGCAAGATACATGGCTCCAGAAGTCCTAGAATCCAGGATG
AATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACTCCATGGCTCTGGTGCTCTG
GGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAGCCTCCATTTGGTT
CCAAGGTGCGGGAGCACCCCTGTGTCGAAAGCATGAAGGACAACGTGTTGAGAGATCGAGGG
CGACCAGAAATTCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGGTGTGTGAGACGTT
GACTGAGTGCTGGGACCACGACCCAGAGGCCCGTCTCACAGCCCAGTGTGTGGCAGAACGCT
TCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGCTGCTCGGAGGAGAAGATTCCT
GAAGACGGCTCCCTAAACACTACCAAA (SEQ ID NO: 44)

ACGATCCCACCGCACGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACAACGG
TGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACC
AGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGTCTGT
GTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACCCCAA
GCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAA
AAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGACAAC
ATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAA
(SEQ ID NO: 45)

ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGC
CAGCACGATCCCACCGCACGTTCAGAAGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAA
TCATCTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATA
GTCACTGACAACAACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATT
TTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGA
AGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACA
GTTTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAA
GTGCATTATGAAGGAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTG
ATGAGTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTG
CTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGT
CATCATCATCTTCTACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAA
CCGGCAAGACGCGGAAGCTCATGGAGTTCAGCGAGCACTGTGCCATCATCCTGGAAGATGAC
CGCTCTGACATCAGCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCCAT
TGAGCTGGACACCCTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGC
AGAACACTTCAGAGCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCC
TCTTGGAAGACAGAGAAGGACATCTTCTCAGACATCAATCTGAAGCATGAGAACATACTCCA
GTTCCTGACGGCTGAGGAGCGGAAGACGGAGTTGGGGAAACAATACTGGCTGATCACCGCCT
TCCACGCCAAGGGCAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGACCTG
CGCAAGCTGGGCAGCTCCCTCGCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATG
TGGGAGGCCCAAGATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGA
ACGACCTAACCTGCTGCCTGTGTGACTTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCT
GTGGATGACCTGGCTAACAGTGGGCAGGTGGGAACTGCAAGATACATGGCTCCAGAAGTCCT
AGAATCCAGGATGAATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACTCCATGG
CTCTGGTGCTCTGGGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAG
CCTCCATTTGGTTCCAAGGTGCGGGAGCACCCCTGTGTCGAAAGCATGAAGGACAACGTGTT
GAGAGATCGAGGGCGACCAGAAATTCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGG
TGTGTGAGACGTTGACTGAGTGCTGGGACCACGACCCAGAGGCCCGTCTCACAGCCCAGTGT
GTGGCAGAACGCTTCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGCTGCTCGGA
GGAGAAGATTCCTGAAGACGGCTCCCTAAACACTACCAAA (SEQ ID NO: 69)

ACGATCCCACCGCACGTTCAGAAGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAATCAT
CTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATAGTCA
CTGACAACAACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCC
ACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCC
ACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTT
GCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGC
ATTATGAAGGAAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGA
GTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAG
TCATATTTCAA (SEQ ID NO: 70).

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGT
CAGCACTGCGGCTGCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGC
AAGACCTTGGGATAGGTGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAA
GGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGG
ATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCA
CTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAAT
GTCAACTTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATT
TAACCGAGATGAGACAATAATCATTGCTTTGGCATCAGTCTCTGTATTAGCTGTTTTGATAG
TTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAAACAAGGTCTTCACAGTATG
AACATGATGGAGGCAGCAGCATCCGAACCCTCTCTTGATCTAGATAATCTGAAACTGTTGGA
GCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTG
CTGTAAAAGTGTTTTCCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGA
GTGCCTTTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGC
AGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATCCCAATGGATCTTTATGCAAGT
ATTTAAGTCTCCACACAAGTGACTGGGTAAGCTCTTGCCGTCTTGCTCATTCTGTTACTAGA
GGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCA
TCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACT
TTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCA
GCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAA
CTTGAGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGACTAATCTATT
GGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATG
GCTTTTCAGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAGGTTCTCGTGTCTAG
GGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCAC
TCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGT
GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGCAACCTGTCACATAATAGGCGTGTGC
CAAAAATTGGTCCTTATCCAGATTATTCTTCCTCCTCATACATTGAAGACTCTATCCATCAT
ACTGACAGCATCGTGAAGAATATTTCCTCTGAGCATTCTATGTCCAGCACACCTTTGACTAT
AGGGGAAAAAAACCGAAATTCAATTAACTATGAACGACAGCAAGCACAAGCTCGAATCCCCA
GCCCTGAAACAAGTGTCACCAGCCTCTCCACCAACACAACAACCACAAACACCACAGGACTC
ACGCCAAGTACTGGCATGACTACTATATCTGAGATGCCATACCCAGATGAAACAAATCTGCA
TACCACAAATGTTGCACAGTCAATTGGGCCAACCCCTGTCTGCTTACAGCTGACAGAAGAAG
ACTTGGAAACCAACAAGCTAGACCCAAAAGAAGTTGATAAGAACCTCAAGGAAAGCTCTGAT
GAGAATCTCATGGAGCACTCTCTTAAACAGTTCAGTGGCCCAGACCCACTGAGCAGTACTAG

TTCTAGCTTGCTTTACCCACTCATAAAACTTGCAGTAGAAGCAACTGGACAGCAGGACTTCA
CACAGACTGCAAATGGCCAAGCATGTTTGATTCCTGATGTTCTGCCTACTCAGATCTATCCT
CTCCCCAAGCAGCAGAACCTTCCCAAGAGACCTACTAGTTTGCCTTTGAACACCAAAAATTC
AACAAAAGAGCCCCGGCTAAAATTTGGCAGCAAGCACAAATCAAACTTGAAACAAGTCGAAA
CTGGAGTTGCCAAGATGAATACAATCAATGCAGCAGAACCTCATGTGGTGACAGTCACCATG
AATGGTGTGGCAGGTAGAAACCACAGTGTTAACTCCCATGCTGCCACAACCCAATATGCCAA
TGGGACAGTACTATCTGGCCAAACAACCAACATAGTGACACATAGGGCCCAAGAAATGTTGC
AGAATCAGTTTATTGGTGAGGACACCCGGCTGAATATTAATTCCAGTCCTGATGAGCATGAG
CCTTTACTGAGACGAGAGCAACAAGCTGGCCATGATGAAGGTGTTCTGGATCGTCTTGTGGA
CAGGAGGGAACGGCCACTAGAAGGTGGCCGAACTAATTCCAATAACAACAACAGCAATCCAT
GTTCAGAACAAGATGTTCTTGCACAGGGTGTTCCAAGCACAGCAGCAGATCCTGGGCCATCA
AAGCCCAGAAGAGCACAGAGGCCTAATTCTCTGGATCTTTCAGCCACAAATGTCCTGGATGG
CAGCAGTATACAGATAGGTGAGTCAACACAAGATGGCAAATCAGGATCAGGTGAAAAGATCA
AGAAACGTGTGAAAACTCCCTATTCTCTTAAGCGGTGGCGCCCCTCCACCTGGGTCATCTCC
ACTGAATCGCTGGACTGTGAAGTCAACAATAATGGCAGTAACAGGGCAGTTCATTCCAAATC
CAGCACTGCTGTTTACCTTGCAGAAGGAGGCACTGCTACAACCATGGTGTCTAAAGATATAG
GAATGAACTGTCTG (SEQ ID NO: 48)

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGG
TGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATG
GCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGGATGTTGGTCTCACATT
GGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCACTCCTCCCTCAATTCA
GAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGAGA
ATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
(SEQ ID NO: 49)

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGT
CAGCACTGCGGCTGCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGC
AAGACCTTGGGATAGGTGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAA
GGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGG
ATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCA
CTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAAT
GTCAACTTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATT
TAACCGAGATGAGACAATAATCATTGCTTTGGCATCAGTCTCTGTATTAGCTGTTTTGATAG
TTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAAACAAGGTCTTCACAGTATG
AACATGATGGAGGCAGCAGCATCCGAACCCTCTCTTGATCTAGATAATCTGAAACTGTTGGA
GCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTG
CTGTAAAAGTGTTTTCCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGA
GTGCCTTTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGC
AGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATCCCAATGGATCTTTATGCAAGT
ATTTAAGTCTCCACACAAGTGACTGGGTAAGCTCTTGCCGTCTTGCTCATTCTGTTACTAGA
GGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCA
TCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACT
TTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCA
GCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAA
CTTGAGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGACTAATCTATT
GGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATG
GCTTTTCAGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAGGTTCTCGTGTCTAG
GGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCAC
TCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGT
GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGTAGG (SEQ ID NO: 73)

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGG
TGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATG
GCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGGATGTTGGTCTCACATT
GGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCACTCCTCCCTCAATTCA
GAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGAGA
ATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
(SEQ ID NO: 74)

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
GCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGC
TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGA
TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGGCT
CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
CTGGATGGCACTGGTGCTGCTGGGGCTGTTCCTCCTCCTCCTGCTGCTGCTGGGCAGCATCA
TCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGAGCCAGTGCCAGAGCCAAGG
CCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCA
GGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACTGGTTG
CCATCAAGGCCTTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTGTACGAA
CTTCCAGGCCTACAGCACGACCACATTGTCCGATTTATCACTGCCAGCCGGGGGGGTCCTGG
CCGCCTGCTCTCTGGGCCCCTGCTGGTACTGGAACTGCATCCCAAGGGCTCCCTGTGCCACT
ACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTGGCCCAG
GGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCA
CCGAGATCTGAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACC
TGGGCCTTGCCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCA
CAAGGCCCAGCTGCCATCATGGAAGCTGGCACCCAGAGGTACATGGCACCAGAGCTCTTGGA
CAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTACTCTTTGG
CTCTGCTCCTGTGGGAGATACTGAGCCGCTGCCCAGATTTGAGGCCTGACAGCAGTCCACCA
CCCTTCCAACTGGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGC
CTTGGCAGTGCAGGAGAGGAGGCGTCCCTACATCCCATCCACCTGGCGCTGCTTTGCCACAG
ACCCTGATGGGCTGAGGGAGCTCCTAGAAGACTGTTGGGATGCAGACCCAGAAGCACGGCTG
ACAGCTGAGTGTGTACAGCAGCGCCTGGCTGCCTTGGCCCATCCTCAAGAGAGCCACCCCTT
TCCAGAGAGCTGTCCACGTGGCTGCCCACCTCTCTGCCCAGAAGACTGTACTTCAATTCCTG
CCCCTACCATCCTCCCCTGTAGGCCTCAGCGGAGTGCCTGCCACTTCAGCGTTCAGCAAGGC
CCTTGTTCCAGGAATCCTCAGCCTGCCTGTACCCTTTCTCCTGTG (SEQ ID NO: 52)

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGAC
ACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCC
GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC
CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
GCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCA
GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 53)

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
GCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGC
TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGA
TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGGCT
CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
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GGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACTGGTTG
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GGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCA
CCGAGATCTGAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACC
TGGGCCTTGCCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCA
CAAGGCCCAGCTGCCATCATGGAAGCTGGCACCCAGAGGTACATGGCACCAGAGCTCTTGGA
CAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTACTCTTTGG
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TCCAACTGGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGCCTTG
GCAGTGCAGGAGAGGAGGCGTCCCTACATCCCATCCACCTGGCGCTGCTTTGCCACAGACCC
TGATGGGC (SEQ ID NO: 77)

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGAC
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GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC
CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
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GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 78)

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
GCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGC
TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGA
TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGGCT
CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
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GGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCA
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CTCCTGTG (SEQ ID NO: 81)

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGAC
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GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC
CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
GCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCA
GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 82)

S

ATGACCTTGGGCTCCCCCAGGAAAGGCCTTCTGATGCTGCTGATGGCCTTGGTGACCCAGGG
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AGGGGCCTACCTGCCGGGGGGCCTGGTGCACAGTAGTGCTGGTGCGGGAGGAGGGGAGGCAC
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CAAGTGGACTGACATCTGGGCCTTTGGCCTGGTGCTGTGGGAGATTGCCCGCCGGACCATCG
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TTTGAGGACATGAAGAAGGTGGTGTGTGTGGATCAGCAGACCCCCACCATCCCTAACCGGCT
GGCTGCAGACCCGGTCCTCTCAGGCCTAGCTCAGATGATGCGGGAGTGCTGGTACCCAAACC
CCTCTGCCCGACTCACCGCGCTGCGGATCAAGAAGACACTACAAAAAATTAGCAACAGTCCA
GAGAAGCCTAAAGTGATTCAA (SEQ ID NO: 16)

GACCCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACCTGCACGTGTGAGAGCCCACATTGCAA
GGGGCCTACCTGCCGGGGGGCCTGGTGCACAGTAGTGCTGGTGCGGGAGGAGGGGAGGCACC
CCCAGGAACATCGGGGCTGCGGGAACTTGCACAGGGAGCTCTGCAGGGGGCGCCCCACCGAG
TTCGTCAACCACTACTGCTGCGACAGCCACCTCTGCAACCACAACGTGTCCCTGGTGCTGGA
GGCCACCCAACCTCCTTCGGAGCAGCCGGGAACAGATGGCCAG (SEQ ID NO: 17)

ATGGTAGATGGAGTGATGATTCTTCCTGTGCTTATCATGATTGCTCTCCCCTCCCCTAGTAT
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CCCGCCGTCCCCTGGCCAAGCCGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACATCA
CGGCCCAGCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAGGTT
GGCCTCATTATTCTCTCTGTAGTGTTCGCAGTATGTCTTTTAGCCTGCCTGCTGGGAGTTGC
TCTCCGAAAATTTAAAAGGCGCAACCAAGAACGCCTCAATCCCCGAGACGTGGAGTATGGCA
CTATCGAAGGGCTCATCACCACCAATGTTGGAGACAGCACTTTAGCAGATTTATTGGATCAT
TCGTGTACATCAGGAAGTGGCTCTGGTCTTCCTTTTCTGGTACAAAGAACAGTGGCTCGCCA
GATTACACTGTTGGAGTGTGTCGGGAAAGGCAGGTATGGTGAGGTGTGGAGGGGCAGCTGGC
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ACGGAATTGTACAACACTGTGATGCTGAGGCATGAAAATATCTTAGGTTTCATTGCTTCAGA
CATGACATCAAGACACTCCAGTACCCAGCTGTGGTTAATTACACATTATCATGAAATGGGAT
CGTTGTACGACTATCTTCAGCTTACTACTCTGGATACAGTTAGCTGCCTTCGAATAGTGCTG
TCCATAGCTAGTGGTCTTGCACATTTGCACATAGAGATATTTGGGACCCAAGGGAAACCAGC
CATTGCCCATCGAGATTTAAAGAGCAAAAATATTCTGGTTAAGAAGAATGGACAGTGTTGCA
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AATCCCCGTGTGGGCACCAAGCGCTACATGGCCCCCGAAGTTCTAGATGAAACCATCCAGGT
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TGGCCAGGCGGATGGTGAGCAATGGTATAGTGGAGGATTACAAGCCACCGTTCTACGATGTG
GTTCCCAATGACCCAAGTTTTGAAGATATGAGGAAGGTAGTCTGTGTGGATCAACAAAGGCC
AAACATACCCAACAGATGGTTCTCAGACCCGACATTAACCTCTCTGGCCAAGCTAATGAAAG
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AAAATTGATAATTCCCTCGACAAATTGAAAACTGACTGT (SEQ ID NO: 20)

ATGGAAGATGAGAAGCCCAAGGTCAACCCCAAACTCTACATGTGTGTGTGTGAAGGTCTCTC
CTGCGGTAATGAGGACCACTGTGAAGGCCAGCAGTGCTTTTCCTCACTGAGCATCAACGATG
GCTTCCACGTCTACCAGAAAGGCTGCTTCCAGGTTTATGAGCAGGGAAAGATGACCTGTAAG
ACCCCGCCGTCCCCTGGCCAAGCCGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACAT
CACGGCCCAGCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAG
(SEQ ID NO: 21)

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   1 ATGCCTCAGC TATACATTTA CATCAGATTA TTGGGAGCCT ATTTGTTCAT CATTTCTCGT
  61 GTTCAAGGAC AGAATCTGGA TAGTATGCTT CATGGCACTG GGATGAAATC AGACTCCGAC
 121 CAGAAAAAGT CAGAAAATGG AGTAACCTTA GCACCAGAGG ATACCTTGCC TTTTTTAAAG
 181 TGCTATTGCT CAGGGCACTG TCCAGATGAT GCTATTAATA ACACATGCAT AACTAATGGA
 241 CATTGCTTTG CCATCATAGA AGAAGATGAC CAGGGAGAAA CCACATTAGC TTCAGGGTGT
 301 ATGAAATATG AAGGATCTGA TTTTCAGTGC AAAGATTCTC CAAAAGCCCA GCTACGCCGG
 361 ACAATAGAAT GTTGTCGGAC CAATTTATGT AACCAGTATT TGCAACCCAC ACTGCCCCCT
 421 GTTGTCATAG GTCCGTTTTT TGATGGCAGC ATTCGATGGC TGGTTTTGCT CATTTCTATG
 481 GCTGTCTGCA TAATTGCTAT GATCATCTTC TCCAGCTGCT TTTGTTACAA ACATTATTGC
 541 AAGAGCATCT CAAGCAGACG TCGTTACAAT CGTGATTTGG AACAGGATGA AGCATTTATT
 601 CCAGTTGGAG AATCACTAAA AGACCTTATT GACCAGTCAC AAAGTTCTGG TAGTGGGTCT
 661 GGACTACCTT TATTGGTTCA GCGAACTATT GCCAAACAGA TTCAGATGGT CCGGCAAGTT
 721 GGTAAAGGCC GATATGGAGA AGTATGGATG GGCAAATGGC GTGGCGAAAA AGTGGCGGTG
 781 AAAGTATTCT TTACCACTGA AGAAGCCAGC TGGTTTCGAG AAACAGAAAT CTACCAAACT
 841 GTGCTAATGC GCCATGAAAA CATACTTGGT TTCATAGCGG CAGACATTAA AGGTACAGGT
 901 TCCTGGACTC AGCTCTATTT GATTACTGAT TACCATGAAA ATGGATCTCT CTATGACTTC
 961 CTGAAATGTG CTACACTGGA CACCAGAGCC CTGCTTAAAT TGGCTTATTC AGCTGCCTGT
1021 GGTCTGTGCC ACCTGCACAC AGAAATTTAT GGCACCCAAG GAAAGCCCGC AATTGCTCAT
1081 CGAGACCTAA AGAGCAAAAA CATCCTCATC AAGAAAAATG GGAGTTGCTG CATTGCTGAC
1141 CTGGGCCTTG CTGTTAAATT CAACAGTGAC ACAAATGAAG TTGATGTGCC CTTGAATACC
1201 AGGGTGGGCA CCAAACGCTA CATGGCTCCC GAAGTGCTGG ACGAAAGCCT GAACAAAAAC
1261 CACTTCCAGC CCTACATCAT GGCTGACATC TACAGCTTCG GCCTAATCAT TTGGGAGATG
1321 GCTCGTCGTT GTATCACAGG AGGGATCGTG GAAGAATACC AATTGCCATA TTACAACATG
1381 GTACCGAGTG ATCCGTCATA CGAAGATATG CGTGAGGTTG TGTGTGTCAA ACGTTTGCGG
1441 CCAATTGTGT CTAATCGGTG GAACAGTGAT GAATGTCTAC GAGCAGTTTT GAAGCTAATG
1501 TCAGAATGCT GGGCCCACAA TCCAGCCTCC AGACTCACAG CATTGAGAAT TAAGAAGACG
1561 CTTGCCAAGA TGGTTGAATC CCAAGATGTA AAAATC  (SEQ ID NO: 24)
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  1  CAGAATCTGG ATAGTATGCT TCATGGCACT GGGATGAAAT CAGACTCCGA CCAGAAAAAG
 61  TCAGAAAATG GAGTAACCTT AGCACCAGAG GATACCTTGC CTTTTTTAAA GTGCTATTGC
121  TCAGGGCACT GTCCAGATGA TGCTATTAAT AACACATGCA TAACTAATGG ACATTGCTTT
181  GCCATCATAG AAGAAGATGA CCAGGGAGAA ACCACATTAG CTTCAGGGTG TATGAAATAT
241  GAAGGATCTG ATTTTCAGTG CAAAGATTCT CCAAAAGCCC AGCTACGCCG GACAATAGAA
301  TGTTGTCGGA CCAATTTATG TAACCAGTAT TTGCAACCCA CACTGCCCCC TGTTGTCATA
361  GGTCCGTTTT TTGATGGCAG CATTCGA  (SEQ ID NO: 25)
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ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGCCGGCAGCGGCG
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ACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACCATGT
GCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTACTGCCTGAGCTCGGA
GGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGATCGACTTGAGGGTGCCCAG
TGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAGCTGGTAGGCATCATCG
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TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTA
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AGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGATCGACTT
GAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAG
(SEQ ID NO: 29)

ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGCCGGCAG
CGGCGGGTCCGGGCCCCGGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGG
CCAACTACACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATG
GAGCACCATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTA
CTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGA
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TCAGCGTGCAGGAAGACGTGAAGATC (SEQ ID NO: 85)

TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTA
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GAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAG
(SEQ ID NO: 86)

ATGGAGGCGGCGGTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGC
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CAACAGGAAGGCATCAAAATG (SEQ ID NO: 32)

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
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TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
GCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
TAAAATAGAACTTCCAACTACTGTAAAGTCATCACCTGGCCTTGGTCCTGTGGAACTG
(SEQ ID NO: 33)

ATGGAGGCGGCGGTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGC
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GACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCC
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ACCATTGCAATAAAATAGAACTTCCAACTACTGGCCCTTTTTCAGTAAAGTCATCACCTGGC
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AGGACCCTTCATTAGATCGCCCTTTTATTTCAGAGGGTACTACGTTGAAAGACTTAATTTAT
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GGCGGGGAGAAGAAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTCGTGGTTCCGT
GAGGCAGAGATTTATCAAACTGTAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGC
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GATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAAACTTGCT
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CTTGTACCTTCTGACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAG
GCCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGA
GAGAATGTTGGTATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATTAAGAAAACATTA
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GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
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CCGGTGGAGCAGTGATGAGTGTCTAAGGCAGATGGGAAAACTCATGACAGAATGCTGGGCTC
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GCACCAAGAAAGAGGATGGTGAGAGTACAGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGT
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TTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAA
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TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAA
AACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATCAC
CAAATGCCCCAAAACTTGGACCCATGGAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTC
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GTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGG
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NO: 40)

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CCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATC
ACCAAATGCCCCAAAACTTGGACCCATGGAG (SEQ ID NO: 41)

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CTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTG
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304)

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TGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCA
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ID NO: 311)

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CCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCCTAA

(SEQ ID NO: 312)

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We claim:

1. A recombinant heteromultimer comprising an ALK3-Fc fusion protein and an ActRIIB-Fc fusion protein,

(a) wherein ALK3-Fc fusion protein comprises:

(1) an ALK3 domain comprising an amino acid sequence that is at least 90% identical to amino acids 61-130 of SEQ ID NO: 22, and

(2) an Fc domain that is an IgG1 Fc domain, and wherein the IgG1 domain comprises a cysteine substitution at the position corresponding to S132 of SEQ ID NO: 3100 (S132C) and a tryptophan substitution at the position corresponding to T144 of SEQ ID NO: 3100 (T144W), wherein the amino acid substitutions alter the isoelectric point (pI) of the ALK3-Fc fusion protein; and

(b) wherein the ActRIIB-Fc fusion protein comprises:

(1) an ActRIIB domain comprising an amino acid sequence that is at least 90% identical to amino acids 29-109 of SEQ ID NO: 1, and

(2) an Fc domain that is an IgG1 Fc domain, and wherein the IgG1 Fc domain comprises one or more amino acid modifications that alter the pI of the ActRIIB-Fc fusion protein.

2. The heteromultimer of claim 1, wherein: the ActRIIB-Fc fusion protein comprises one or more amino acid modifications that decrease the pI of the ActRIIB-Fc fusion protein.

3. The heteromultimer of claim 2, wherein the ActRIIB-Fc fusion protein comprises one or more neutral or positively charged amino acid substitutions with one or more negatively charged amino acids.

4. The heteromultimer of any one of claims 1-3, wherein the ActRIIB-Fc fusion protein IgG1 Fc domain comprises a cysteine substitution at the position corresponding to Y127 of SEQ ID NO: 3100 (Y127C), a serine substitution at the position corresponding to T144 of SEQ ID NO: 3100 (T144S), an alanine substitution at the position corresponding to L146 of SEQ ID NO: 3100 (L146A), and a valine substitution at the position corresponding to Y185 of SEQ ID NO: 3100 (Y185V).

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5. The heteromultimer of any one of the preceding claims, wherein the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence selected from:

a) a polypeptide that:

i) begins at any one of amino acids of 24-61 (e.g., amino acid residues 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, and 61) SEQ ID NO: 22, and

ii) ends at any one of amino acids 130-152 (e.g., amino acid residues 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, and 152) of SEQ ID NO: 22;

b) amino acids 24-152 of SEQ ID NO: 22; and

c) amino acid sequence of any one of SEQ ID Nos: 22, 23, 115, 117, 407, and 408.

6. The heteromultimer of any one of the preceding claims, wherein the ActRII-Fc fusion protein comprises an ActRIIB domain comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence selected from:

a) a polypeptide that:

i) begins at any one of amino acids of 20-29 (e.g., amino acid residues 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) SEQ ID NO: 1, and

ii) ends at any one of amino acids 109-134 (e.g., amino acid residues 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134 of SEQ ID NO: 1;

b) 20-134 of SEQ ID NO: 1;

c) 25-131 of SEQ ID NO: 1; and

d) amino acid sequence of any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 401, and 402.

7. The heteromultimer of any one of the preceding claims, wherein the ALK3-Fc fusion protein further comprises a linker domain positioned between the ALK3 domain and the Fc

domain, and wherein the ActRIIB-Fc fusion protein further comprises a linker domain positioned between the ActRIIB domain and the Fc domain.

8. The heteromultimer of claim 7, wherein the linker domain is selected from: TGGG (SEQ ID NO: 62), TGGGG (SEQ ID NO: 60), SGGGG (SEQ ID NO: 61), GGGGS, GGG (SEQ ID NO: 58), GGGG (SEQ ID NO: 59), and SGGG (SEQ ID NO: 18).

9. The heteromultimer of any one of the preceding claims, wherein the heteromultimer is a heterodimer.

10. The heteromultimer of any one of the preceding claims, wherein the ALK3-Fc fusion protein and the ActRIIB-Fc fusion protein have at least a 0.7 difference in pI.

11. A pharmaceutical preparation comprising the heteromultimer of any one of the preceding claims and a pharmaceutically acceptable carrier.

12. A recombinant nucleic acid comprising a coding sequence for an ALK3-Fc fusion protein as described in any one of the preceding claims.

13. A recombinant nucleic acid comprising a coding sequence for an ActRIIB-Fc fusion protein as described in any one of the preceding claims.

14. A recombinant polynucleotide comprising a promoter sequence operably linked to the recombinant nucleic acid of claim 12 and the recombinant nucleic acid of claim 13.

15. A cell comprising either the recombinant nucleic acid of claim 12 and/or claim 13, or the recombinant polynucleotide of claim 14.

16. The cell of claim 15, wherein the cell is a CHO cell.

17. A method of making a heteromultimer comprising an ALK3-Fc fusion protein and a ActRIIB-Fc fusion protein comprising culturing a cell under conditions suitable for expression of an ALK3-Fc fusion protein and an ActRIIB-Fc fusion protein, wherein the cell comprises the recombinant nucleic acid of claim 12 and claim 13.

18. A method of making a heteromultimer comprising an ALK3-Fc fusion protein and a ActRIIB-Fc fusion protein comprising culturing a cell under conditions suitable for expression of an ALK3-Fc fusion protein and an ActRIIB-Fc fusion protein, wherein the cell comprises the recombinant polynucleotide of claim 14.

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19. A method of making a heteromultimer comprising an ALK3-Fc fusion protein and a ActRIIB-Fc fusion protein comprising:

a) culturing a first cell under conditions suitable for expression of an ALK3-Fc fusion protein, wherein the first cell comprises the recombinant polynucleotide of claim 12;

b) recovering the ALK3-Fc fusion protein so expressed;

c) culturing a second cell under conditions suitable for expression of a ActRIIB-Fc fusion protein, wherein the second cell comprises the recombinant polynucleotide of claim 13;

d) recovering the ActRIIB-Fc fusion protein so expressed;

e) combining the recovered ALK3-Fc fusion protein and the recovered ActRIIB-Fc fusion protein under conditions suitable for type I:type II heteromultimer formation.

20. The method of claim 19, wherein the ALK3-Fc fusion protein and/or the ActRIIB-Fc fusion protein are expressed using a TPA leader sequence, and wherein the TPA leader comprises a coding sequence for SEQ ID NO: 98.

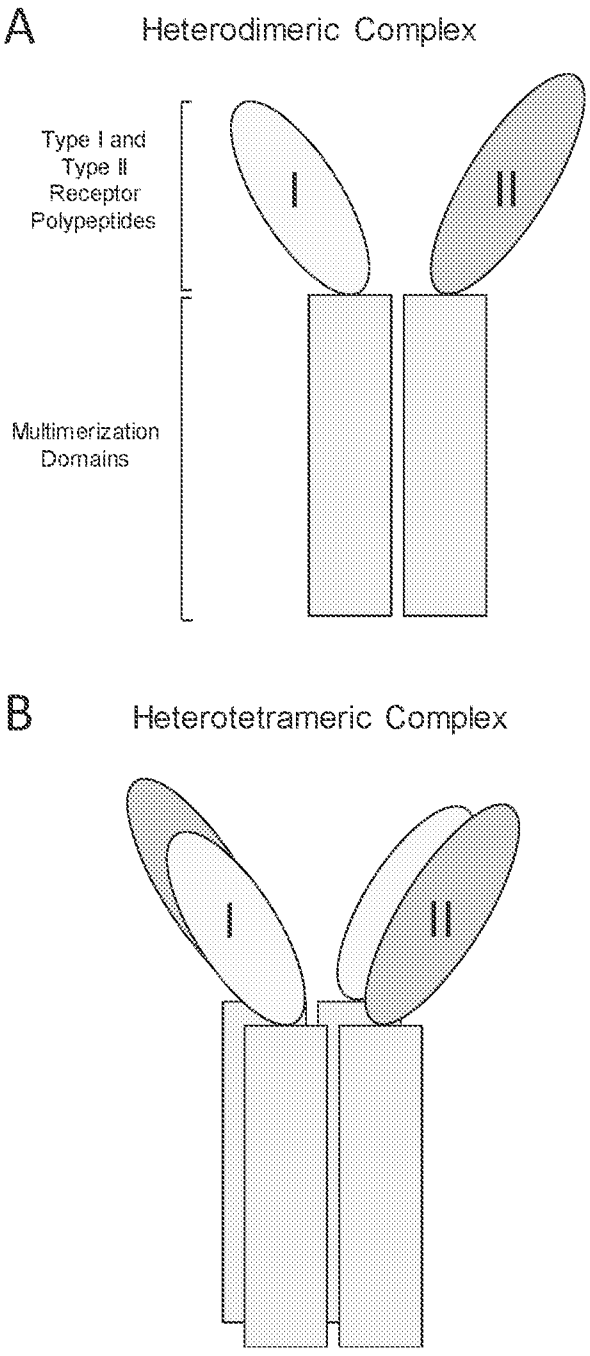

A
Heterodimeric Complex
Type I and
Type II
Receptor
Polypeptides
I
II
Multimerization
Domains
B
Heterotetrameric Complex
I
II

FIGURE 1

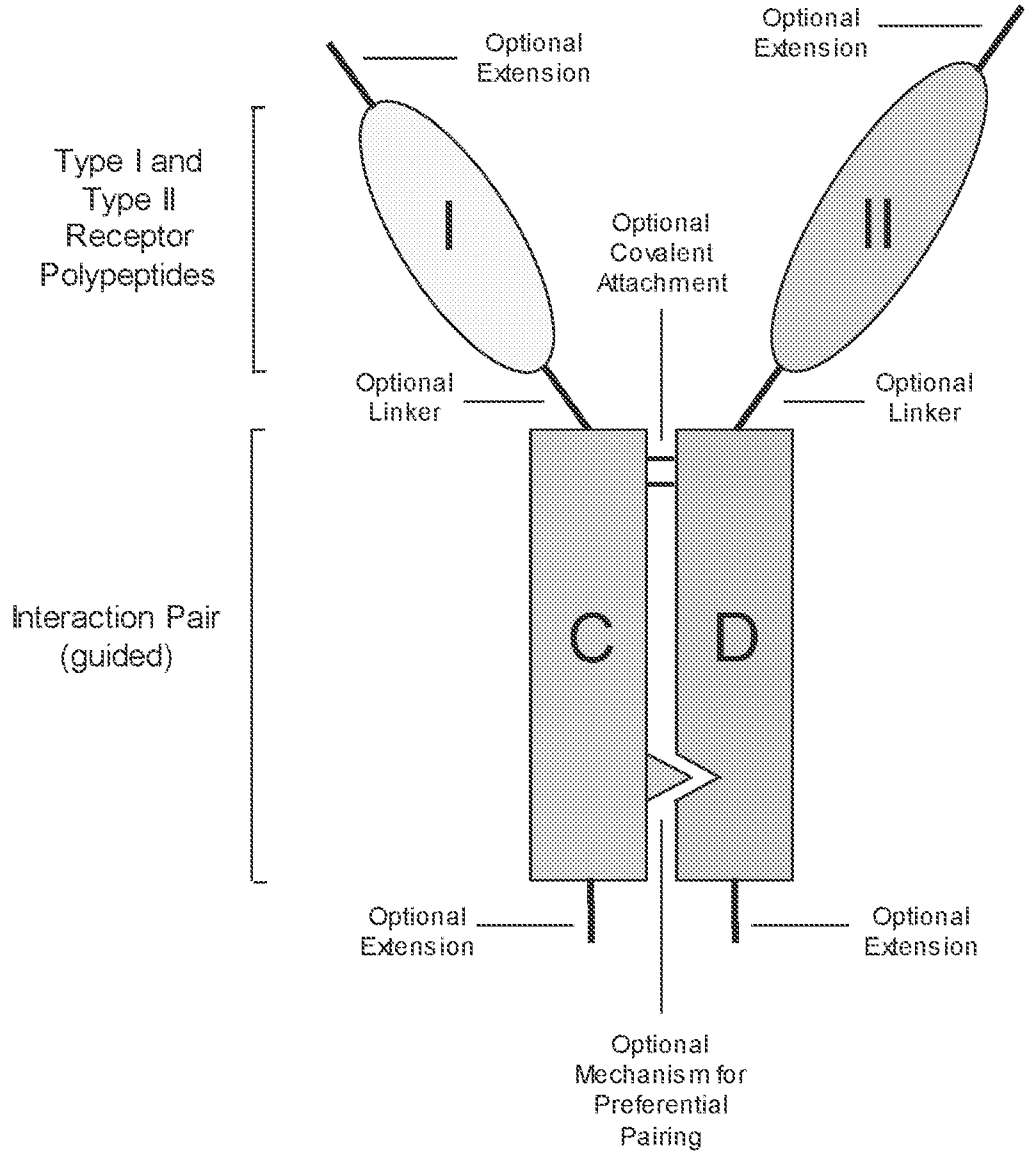

Schematic Heteromeric Protein Complex
Optional Extension
Optional Extension
Type I and Type II Receptor Polypeptides
I
II
Optional Covalent Attachment
Optional Linker
Optional Linker
Interaction Pair (guided)
C
D
Optional Extension
Optional Extension
Optional Mechanism for Preferential Pairing

FIGURE 2

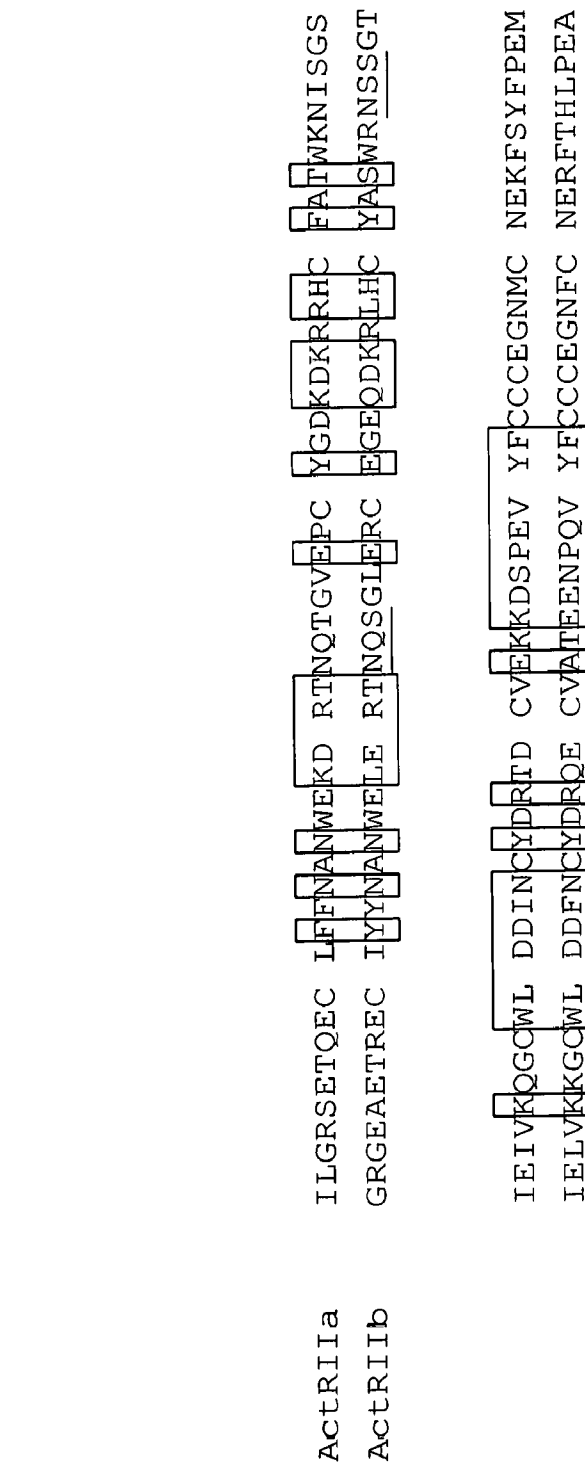

ActRIIa
ActRIIb
ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
EVTQPTSNPV TPKPPT
GGPEVTYEPP PTAPT

FIGURE 3

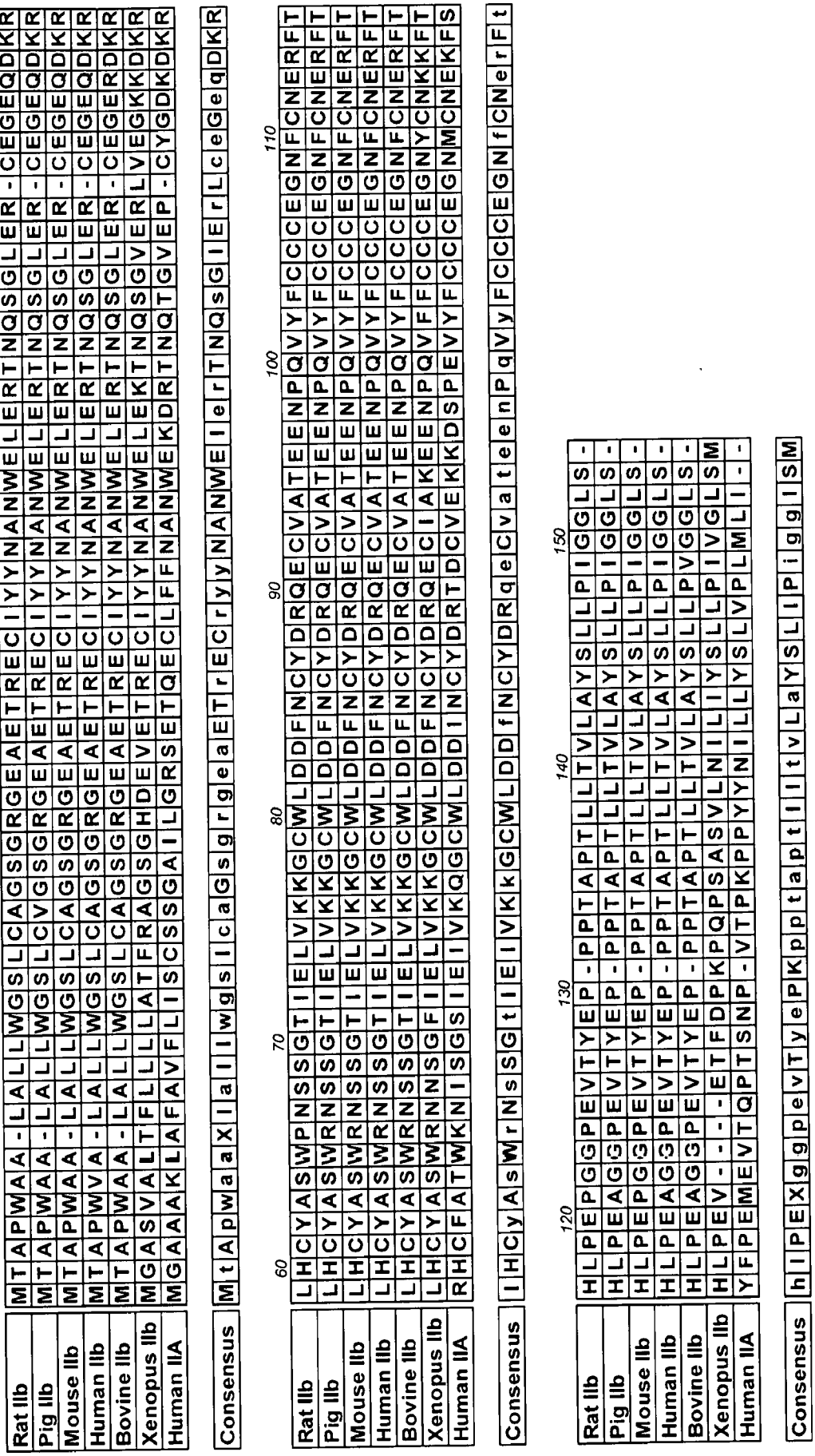

Rat IIb
Pig IIb
Mouse IIb
Human IIb
Bovine IIb
Xenopus IIb
Human IIA
Consensus

FIGURE 4

```
IgG1  -------THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF   53
IgG4  ---ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF   57
IgG2  --------VECPPCPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF   51
IgG3  EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF   60
                ** ****  .  * ******************************:*****:*

IgG1  NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT   113
IgG4  NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT   117
IgG2  NWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT   111
IgG3  KWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT   120
      :*******************:***:********:*****************.**:.****

IgG1  ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP   173
IgG4  ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP   177
IgG2  ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP   171
IgG3  ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTP   180
      ***:***************:****************************.*******:***

IgG1  PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK    225
IgG4  PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK    229
IgG2  PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK    223
IgG3  PMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK    232
      *:***********:*********:**:************::******** **
```

FIGURE 5

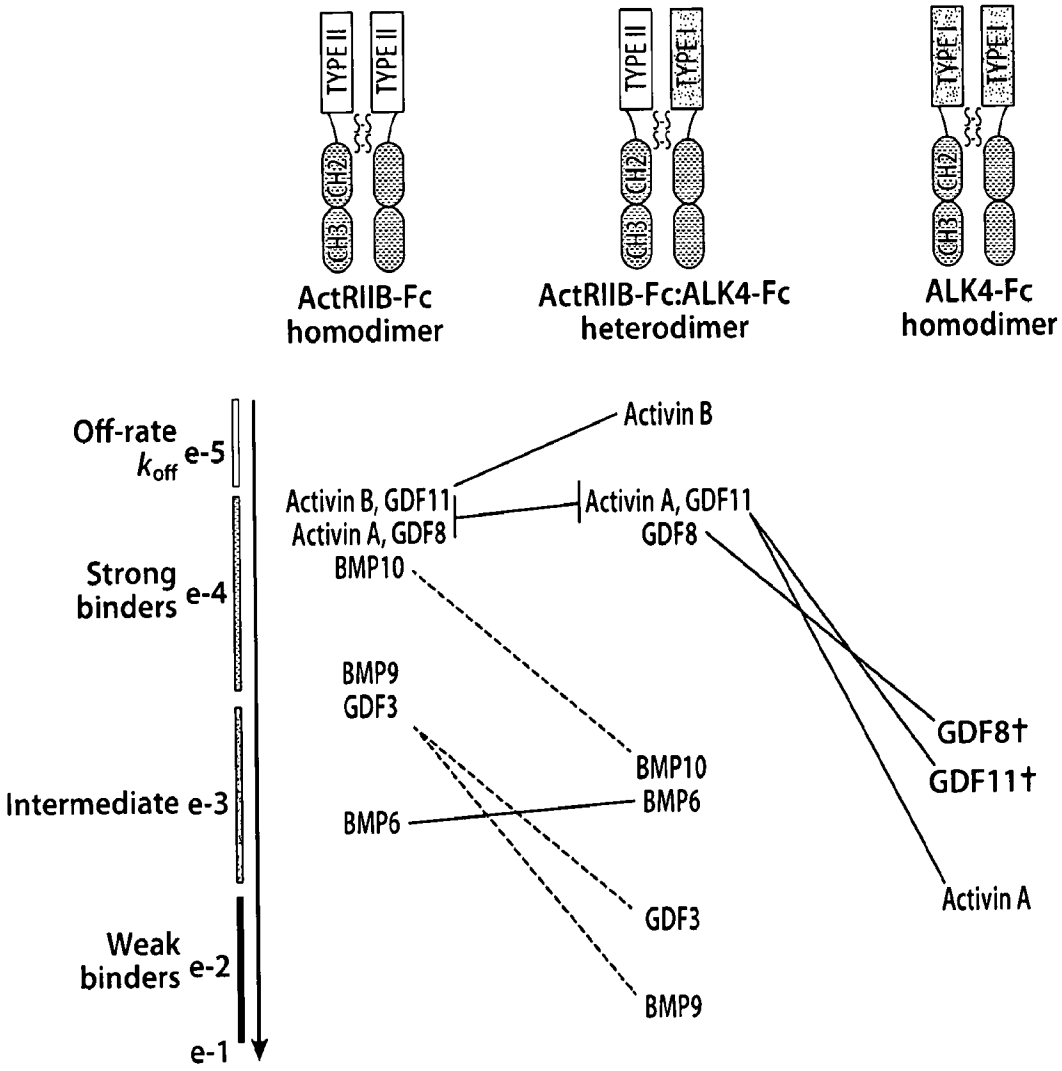

TYPE II
TYPE II
CH2
CH3
ActRIIB-Fc homodimer
TYPE II
TYPE I
ActRIIB-Fc:ALK4-Fc heterodimer
TYPE I
TYPE I
ALK4-Fc homodimer
Off-rate k_{off} e-5
Strong binders e-4
Intermediate e-3
Weak binders e-2
e-1
Activin B
Activin B, GDF11
Activin A, GDF8
Activin A, GDF11
GDF8
BMP10
BMP9
GDF3
BMP6
BMP10
BMP6
GDF3
BMP9
GDF8†
GDF11†
Activin A

FIGURE 6

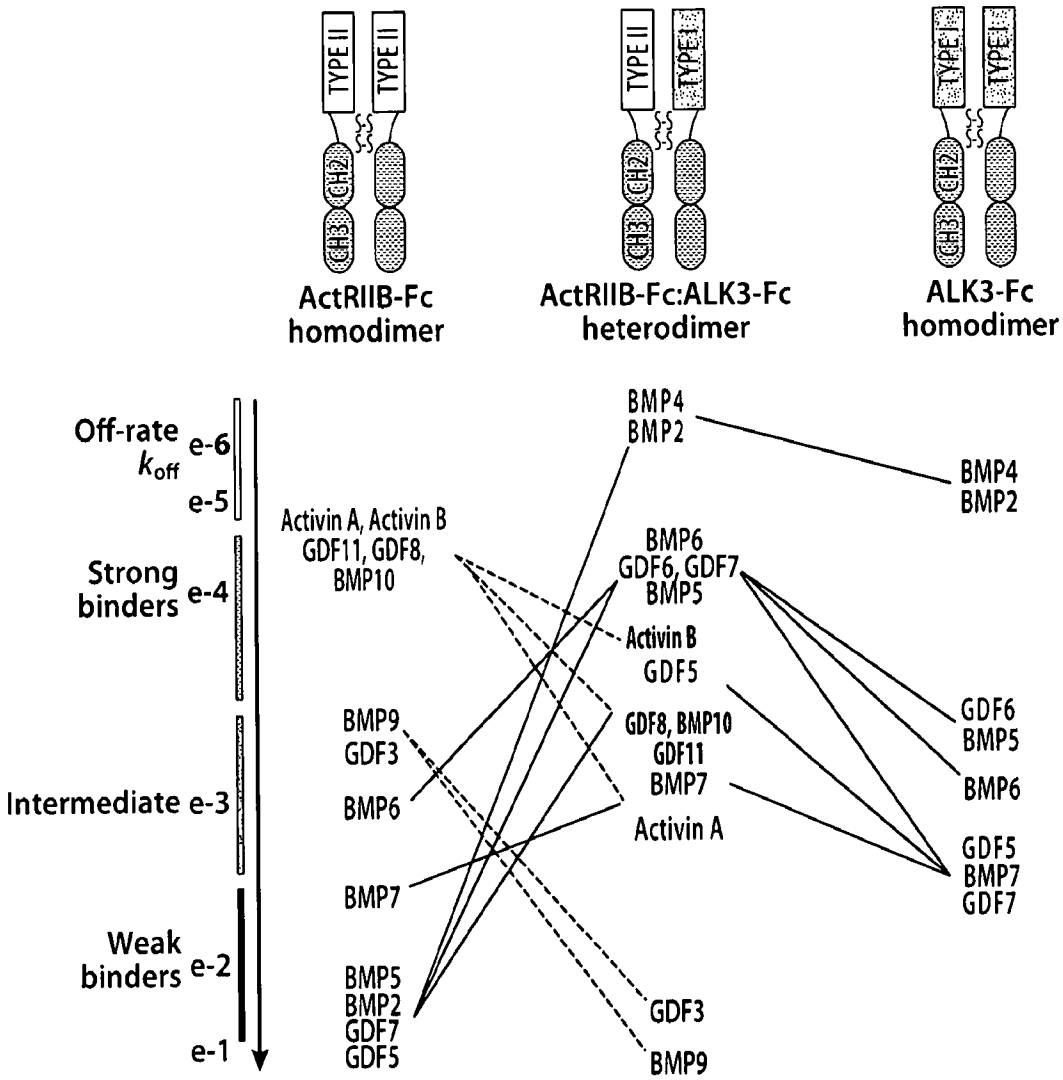

TYPE II
TYPE II
CH2
CH3
ActRIIB-Fc homodimer
TYPE II
TYPE I
ActRIIB-Fc:ALK3-Fc heterodimer
TYPE I
TYPE I
ALK3-Fc homodimer
Off-rate k_{off}
e-6
e-5
Strong binders e-4
Intermediate e-3
Weak binders e-2
e-1
Activin A, Activin B GDF11, GDF8, BMP10
BMP9 GDF3
BMP6
BMP7
BMP5 BMP2 GDF7 GDF5
BMP4 BMP2
BMP6 GDF6, GDF7 BMP5
Activin B GDF5
GDF8, BMP10 GDF11 BMP7
Activin A
GDF3
BMP9
BMP4 BMP2
GDF6 BMP5
BMP6
GDF5 BMP7 GDF7

FIGURE 7

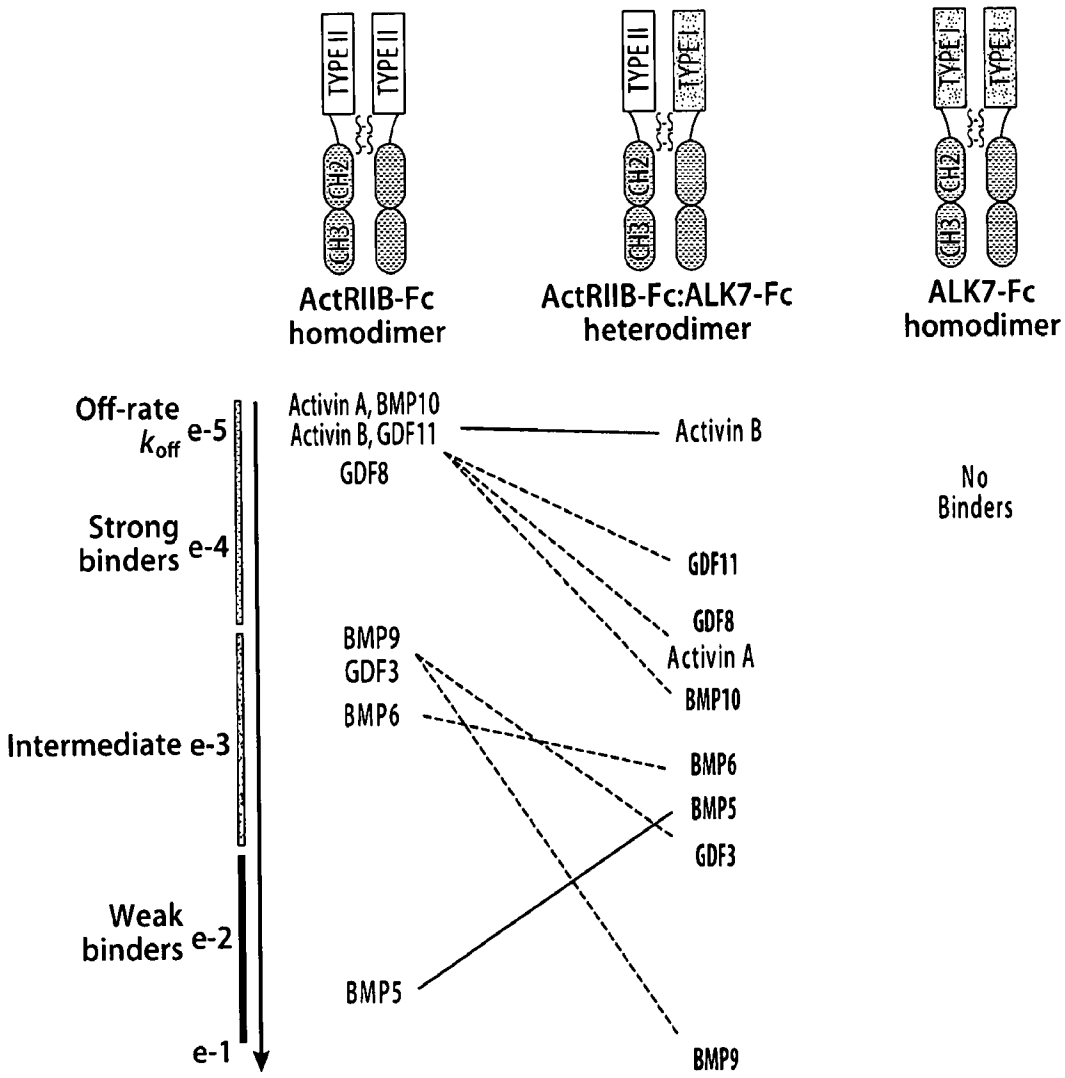

TYPE II
TYPE II
CH2
CH3
ActRIIB-Fc homodimer
TYPE II
TYPE I
ActRIIB-Fc:ALK7-Fc heterodimer
TYPE I
TYPE I
ALK7-Fc homodimer
Off-rate k_{off}
e-5
Strong binders
e-4
Intermediate
e-3
Weak binders
e-2
e-1
Activin A, BMP10
Activin B, GDF11
GDF8
BMP9
GDF3
BMP6
BMP5
Activin B
GDF11
GDF8
Activin A
BMP10
BMP6
BMP5
GDF3
BMP9
No Binders

FIGURE 8

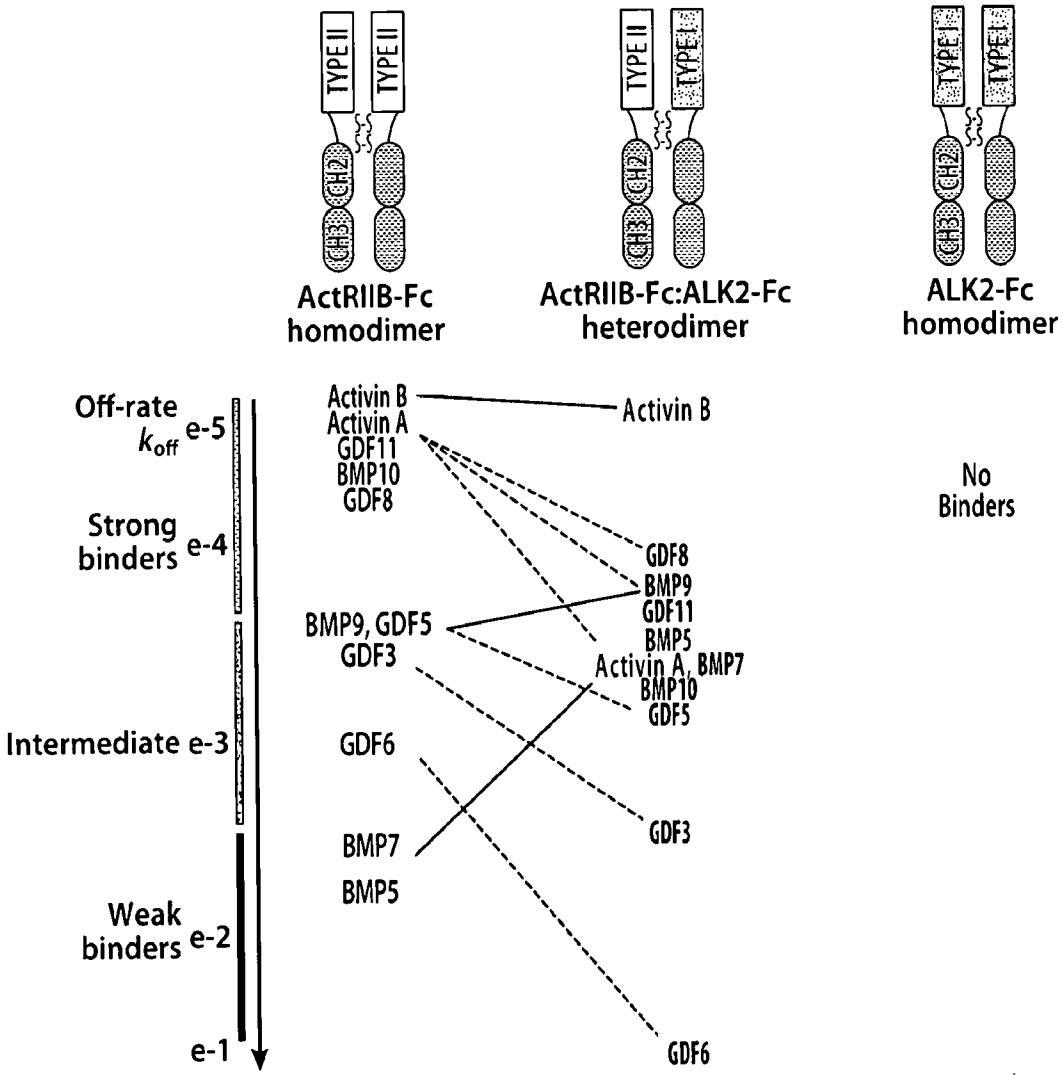

TYPE II
TYPE II
CH2
CH3
ActRIIB-Fc homodimer
TYPE II
TYPE I
ActRIIB-Fc:ALK2-Fc heterodimer
TYPE I
TYPE I
ALK2-Fc homodimer
Off-rate k_{off} e-5
Strong binders e-4
Intermediate e-3
Weak binders e-2
e-1
Activin B
Activin A
GDF11
BMP10
GDF8
Activin B
GDF8
BMP9
GDF11
BMP5
Activin A, BMP7
BMP10
GDF5
BMP9, GDF5
GDF3
GDF6
BMP7
BMP5
GDF3
GDF6
No Binders

FIGURE 9

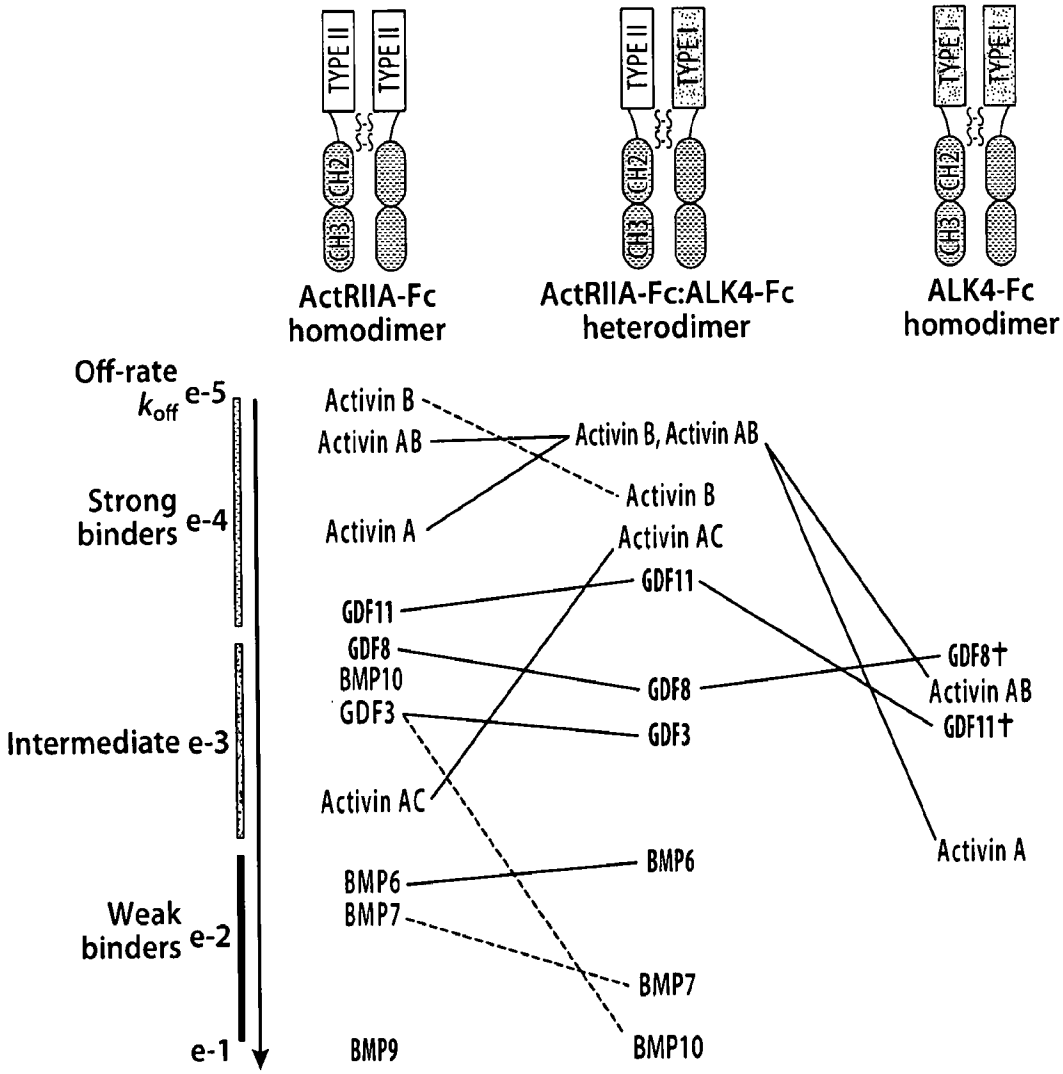

TYPE II
TYPE II
CH2
CH3
ActRIIA-Fc homodimer
TYPE II
TYPE I
ActRIIA-Fc:ALK4-Fc heterodimer
TYPE I
TYPE I
ALK4-Fc homodimer
Off-rate k_{off}
e-5
Strong binders
e-4
Intermediate
e-3
Weak binders
e-2
e-1
Activin B
Activin AB
Activin A
Activin B, Activin AB
Activin B
Activin AC
GDF11
GDF11
GDF8
BMP10
GDF3
GDF8
GDF3
Activin AC
BMP6
BMP6
BMP7
BMP7
BMP9
BMP10
GDF8†
Activin AB
GDF11†
Activin A

FIGURE 10

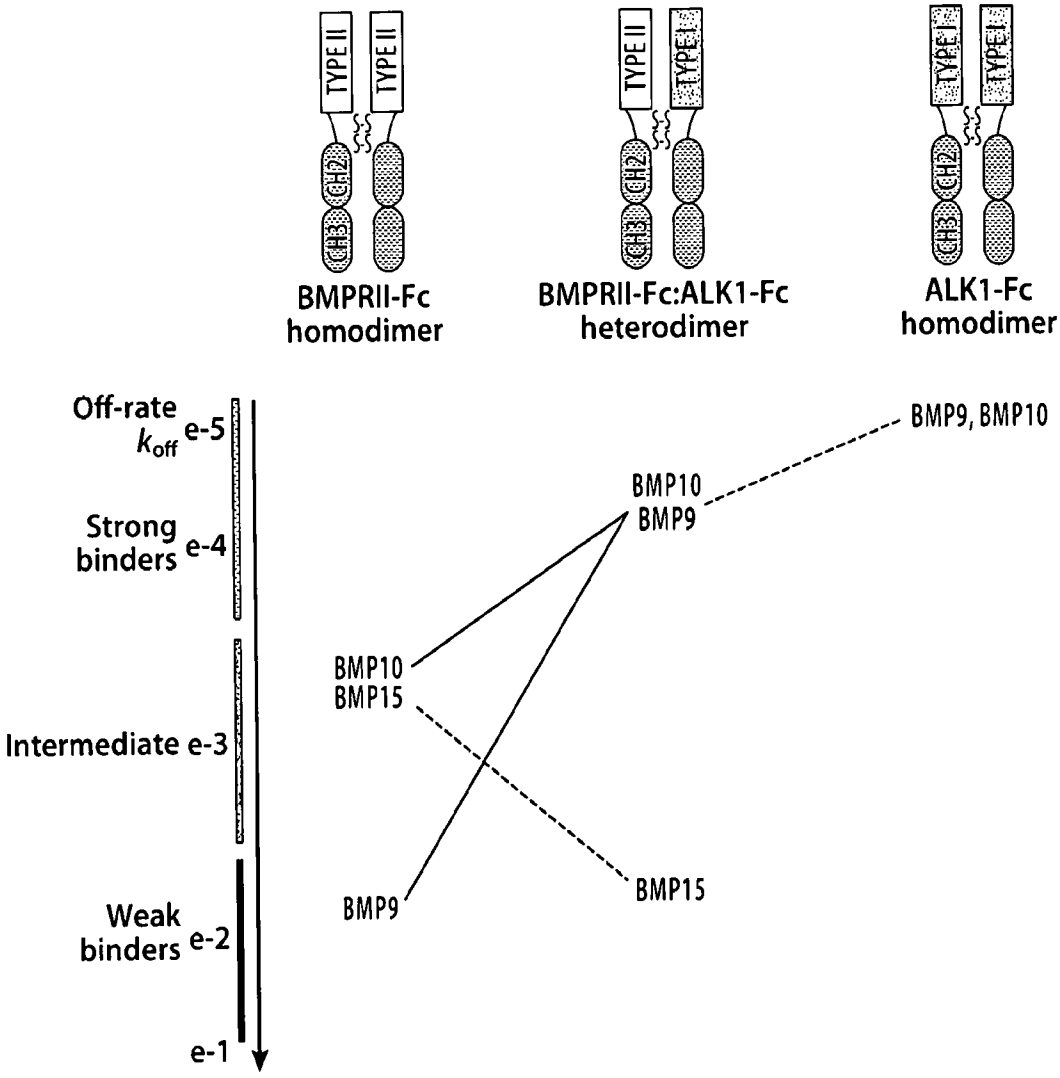

TYPE II
TYPE II
CH2
CH3
BMPRII-Fc
homodimer
TYPE II
TYPE I
BMPRII-Fc:ALK1-Fc
heterodimer
TYPE I
TYPE I
ALK1-Fc
homodimer
Off-rate
k_{off}
e-5
Strong
binders
e-4
Intermediate
e-3
Weak
binders
e-2
e-1
BMP9, BMP10
BMP10
BMP9
BMP10
BMP15
BMP15
BMP9

FIGURE 11

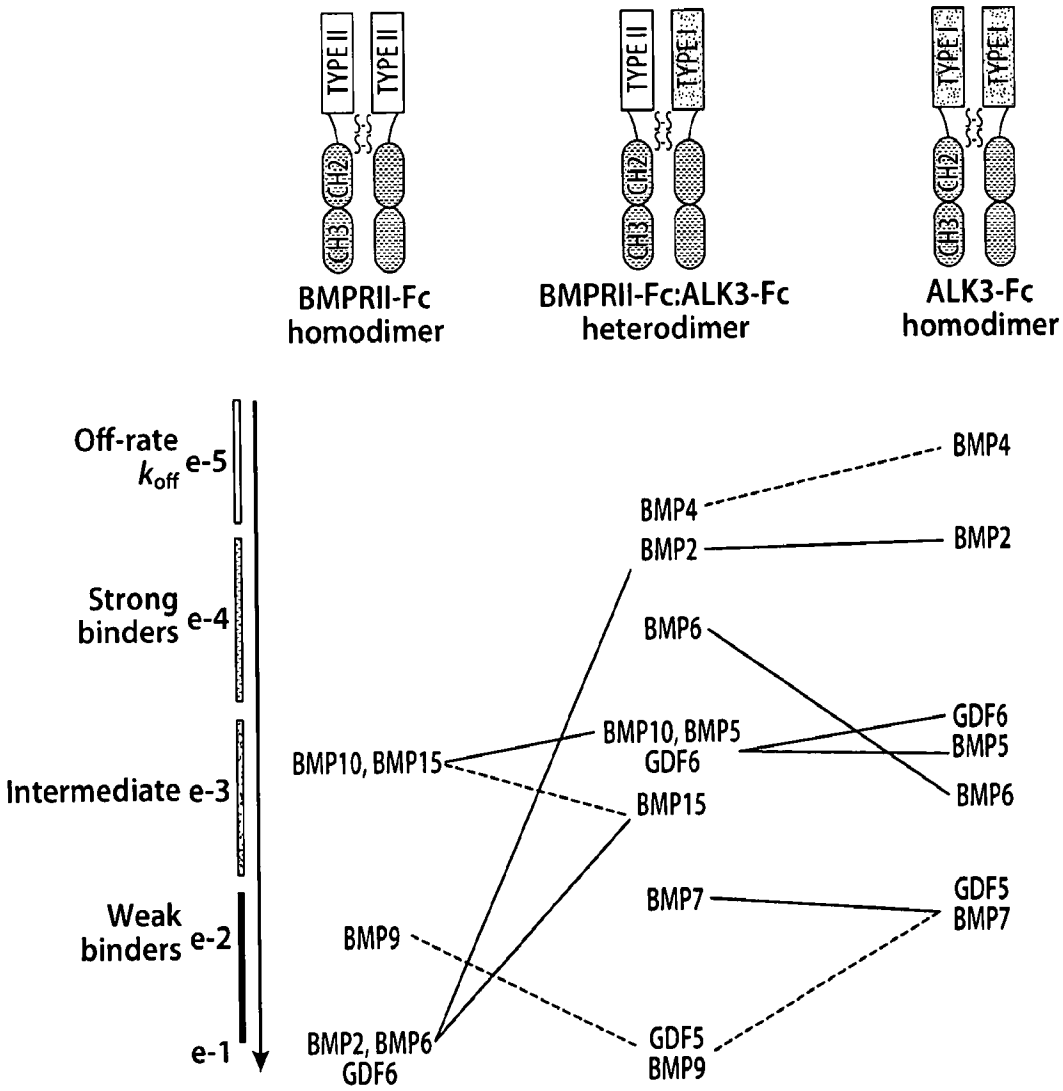

TYPE II
TYPE II
CH2
CH3
BMPRII-Fc homodimer
TYPE II
TYPE I
BMPRII-Fc:ALK3-Fc heterodimer
TYPE I
TYPE I
ALK3-Fc homodimer
Off-rate k_{off} e-5
Strong binders e-4
Intermediate e-3
Weak binders e-2
e-1
BMP4
BMP2
BMP6
BMP10, BMP5
GDF6
BMP10, BMP15
BMP15
BMP7
BMP9
BMP2, BMP6
GDF6
GDF5
BMP9
BMP4
BMP2
GDF6
BMP5
BMP6
GDF5
BMP7

FIGURE 12

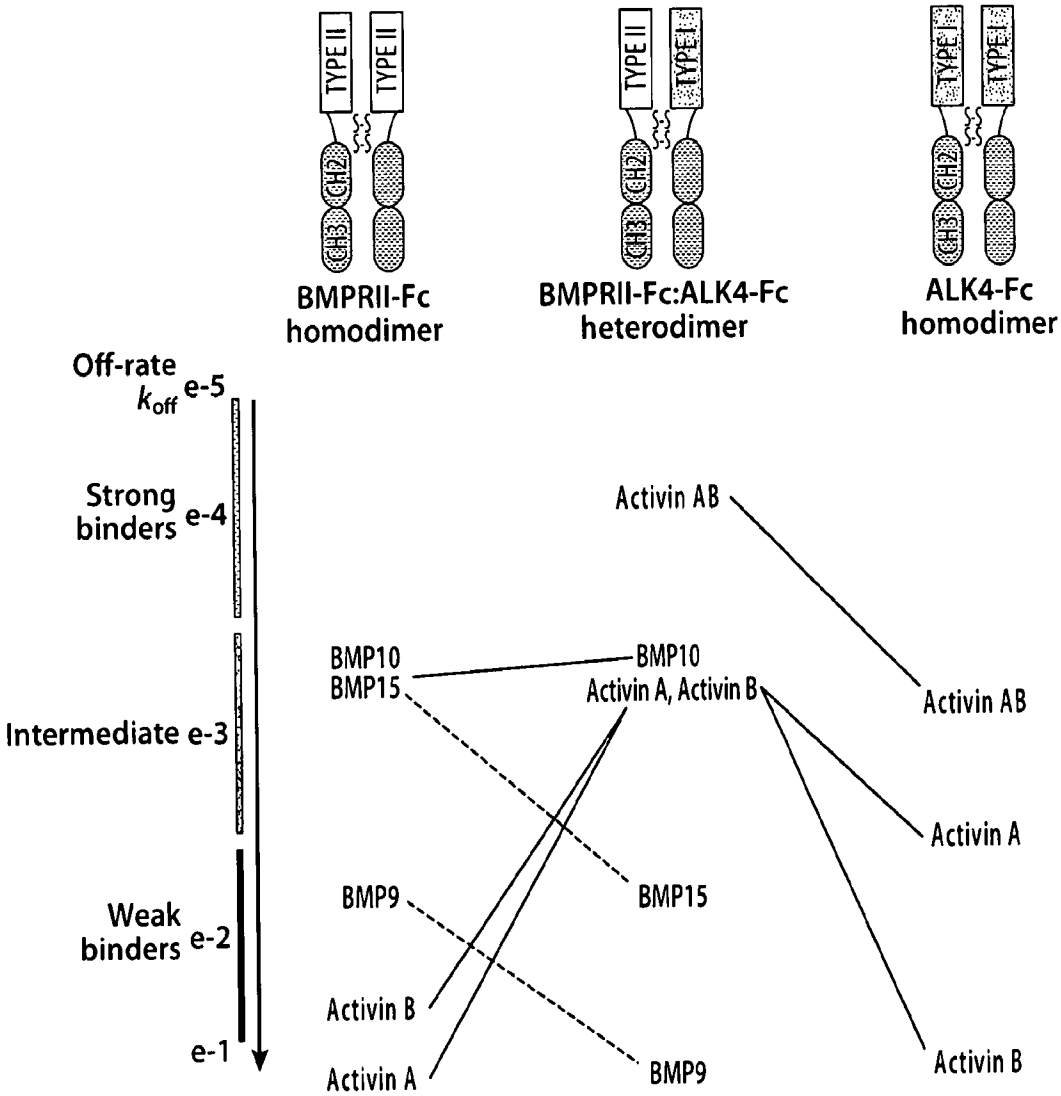

TYPE II
TYPE II
CH2
CH3
BMPRII-Fc homodimer
TYPE II
TYPE I
BMPRII-Fc:ALK4-Fc heterodimer
TYPE I
TYPE I
ALK4-Fc homodimer
Off-rate k_{off} e-5
Strong binders e-4
Intermediate e-3
Weak binders e-2
e-1
BMP10
BMP15
BMP9
Activin B
Activin A
BMP10
Activin A, Activin B
BMP15
BMP9
Activin AB
Activin AB
Activin A
Activin B

FIGURE 13

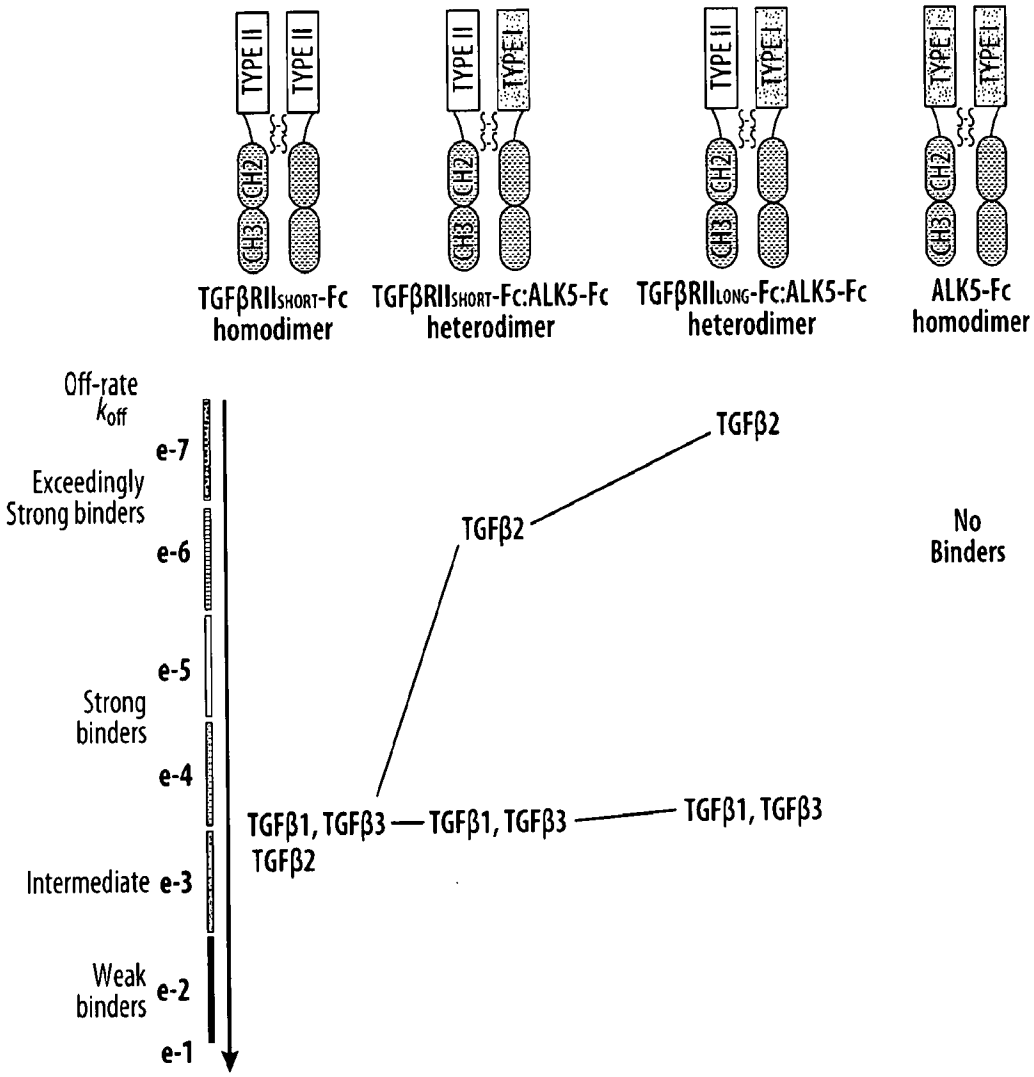

TYPE II
TYPE II
CH2
CH3
TGFβRIIsHORT-Fc homodimer
TYPE II
TYPE I
TGFβRIIsHORT-Fc:ALK5-Fc heterodimer
TYPE II
TYPE I
TGFβRIILONG-Fc:ALK5-Fc heterodimer
TYPE I
TYPE I
ALK5-Fc homodimer
Off-rate koff
e-7
Exceedingly Strong binders
e-6
e-5
Strong binders
e-4
Intermediate e-3
Weak binders e-2
e-1
TGFβ2
TGFβ2
No Binders
TGFβ1, TGFβ3
TGFβ1, TGFβ3
TGFβ1, TGFβ3
TGFβ2

FIGURE 14

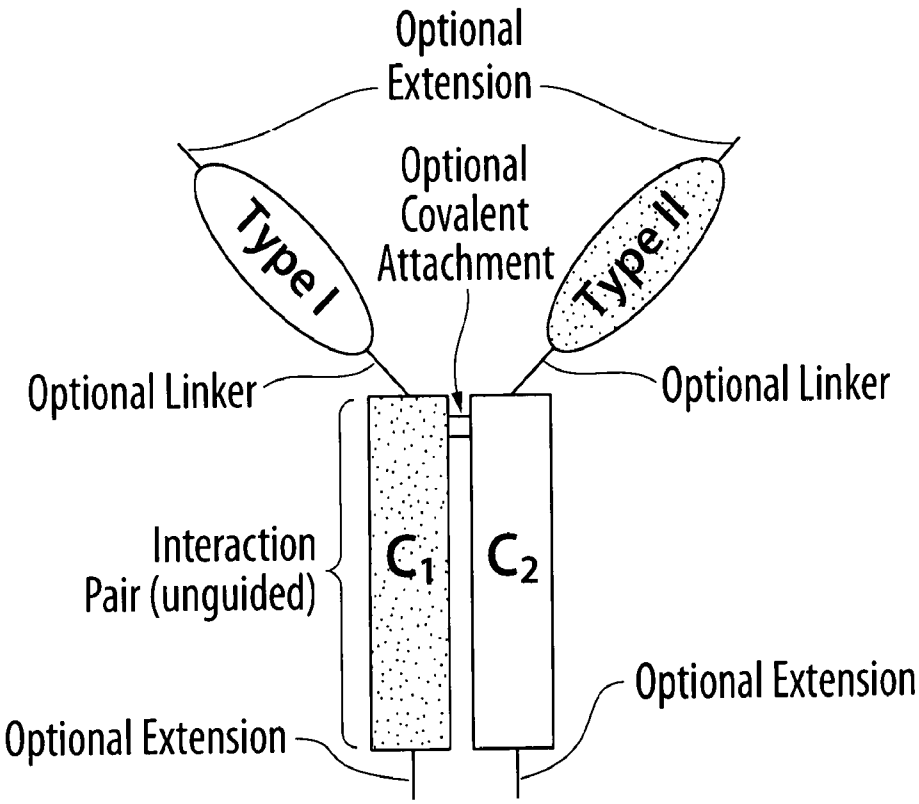

Optional
Extension
Optional
Covalent
Attachment
Type I
Type II
Optional Linker
Optional Linker
Interaction
Pair (unguided)
C1
C2
Optional Extension
Optional Extension

FIGURE 15A

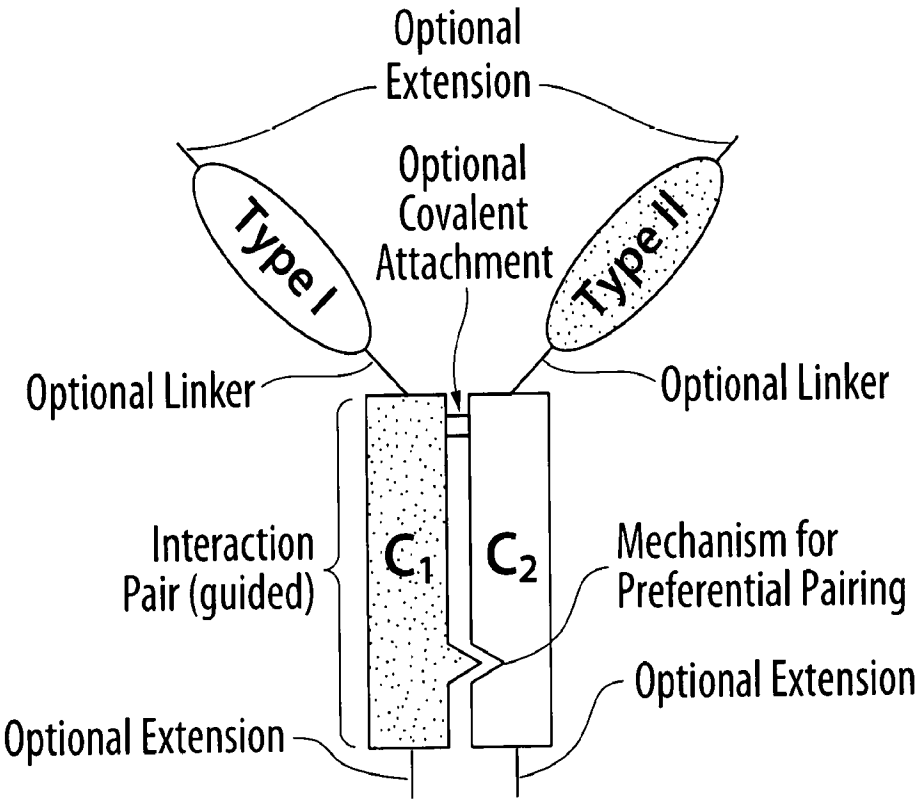

Optional
Extension
Optional
Covalent
Attachment
Type I
Type II
Optional Linker
Optional Linker
Interaction
Pair (guided)
C1
C2
Mechanism for
Preferential Pairing
Optional Extension
Optional Extension

FIGURE 15B

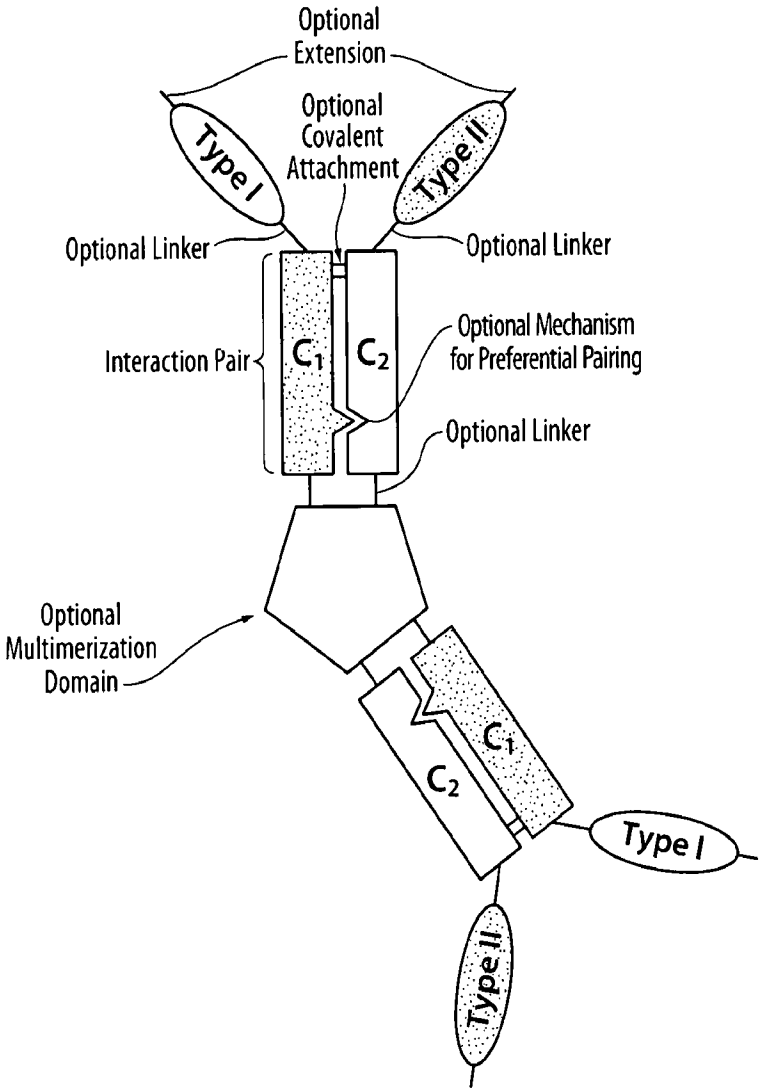

Optional
Extension
Optional
Covalent
Attachment
Type I
Type II
Optional Linker
Optional Linker
Interaction Pair
C1
C2
Optional Mechanism
for Preferential Pairing
Optional Linker
Optional
Multimerization
Domain
C1
C2
Type I
Type II

FIGURE 15C

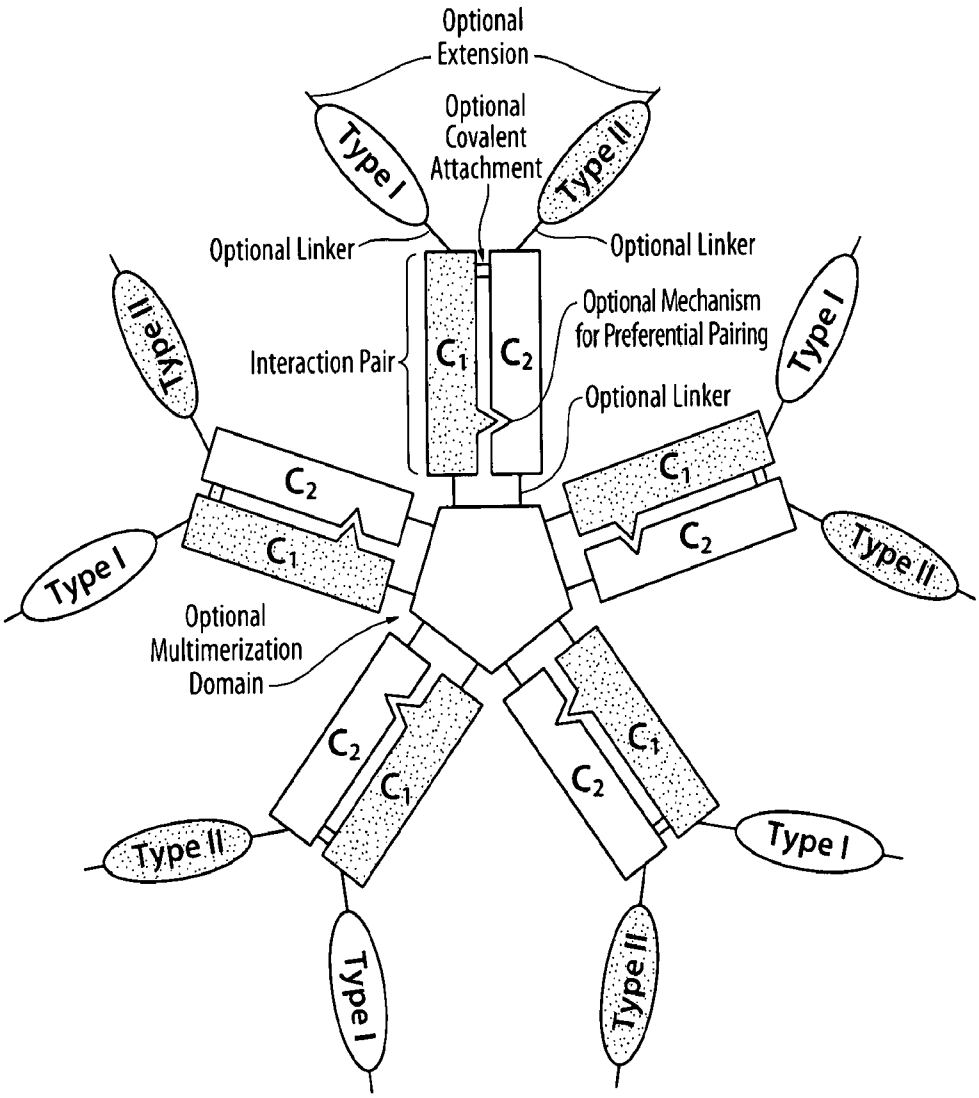

Optional Extension
Optional Covalent Attachment
Type I
Type II
Optional Linker
Optional Linker
Optional Mechanism for Preferential Pairing
Interaction Pair
C1
C2
Optional Linker
Optional Multimerization Domain

FIGURE 15D