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(71) Applicant(s)
Acceleron Pharma Inc.

(72) Inventor(s)
Kumar, Ravindra;Grinberg, Asya;Sako, Dianne S.

(74) Agent / Attorney
Shelston IP Pty Ltd., L 9 60 Margaret St, Sydney, NSW, 2000, AU

(56) Related Art
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(71) Applicant: **ACCELERON PHARMA INC.** [US/US];
128 Sidney Street, Cambridge, MA 02139 (US).

(72) Inventors: **KUMAR, Ravindra**; 421 Arlington Street, Ac-
ton, MA 01720 (US). **GRINBERG, Asya**; 37 Follen
Road, Lexington, MA 02421 (US). **SAKO, Dianne, S.**; 14
Mystic Street, Medford, MA 02155 (US).

(74) Agents: **VARMA, Anita** et al.; Ropes & Gray LLP,
Prudential Tower, 800 Boylston Street, Boston, MA 02199
(US).

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(54) Title: SINGLE-ARM TYPE I AND TYPE II RECEPTOR FUSION PROTEINS AND USES THEREOF

(57) Abstract: In certain aspects, the disclosure provides soluble single-arm heteromeric polypeptide complexes comprising an ex-
tracellular domain of a type I serine/threonine kinase receptor of the TGF-beta family or an extracellular domain of a type II
serine/threonine kinase receptor of the TGF-beta family. In some embodiments, the disclosure provides soluble single-arm poly-
peptide complexes comprising an extracellular domain of a type II receptor selected from: ActRIIA, ActRIIB, TGFBR2, BMPRII,
and MISRII. In some embodiments, the disclosure provides soluble single-arm polypeptide complexes comprising an extracellular
domain of a type I receptor selected from: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7. Optionally the soluble complex
is a heterodimer. In certain aspects, such soluble polypeptide complexes may be used for the treatment or prevention of various
TGF-beta associated conditions, including without limitation diseases and disorders associated with, for example, cancer, muscle,
bone, fat, red blood cells, metabolism, fibrosis and other tissues that are affected by one or more ligands of the TGF-beta superfam-
ily.



SINGLE-ARM TYPE I AND TYPE II RECEPTOR FUSION PROTEINS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of priority to United States Provisional Application Serial Nos. 62/143,579, filed April 6, 2015, and 62/259,422, filed November 24, 2015. The disclosures of the foregoing applications are hereby incorporated by reference in their entirety

BACKGROUND OF THE INVENTION

10 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
15 differentiation processes, including adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. The superfamily 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
20 GDFs. Hinck (2012) FEBS Letters 586:1860-1870. TGF-beta superfamily members have diverse, often complementary biological effects. By manipulating the activity of a member of the TGF-beta superfamily, 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
25 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
30 of the TGF-beta superfamily. Thus, there is a need for agents that regulate the activity of various ligands of the TGF-beta superfamily.

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 the 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

According to a first aspect, the present invention provides a soluble protein complex comprising a first polypeptide covalently or non-covalently associated with a second polypeptide, wherein:

- a. the first polypeptide comprises an amino acid sequence of a first member of an interaction pair and an amino acid sequence of an ActRIIB polypeptide, which is at least 85% identical to amino acids 29-109 of SEQ ID NO: 1; and wherein the ActRIIB polypeptide comprises a leucine at the amino acid position corresponding to position 79 of SEQ ID NO: 1; and
- b. the second polypeptide comprises an amino acid sequence of a second member of the interaction pair, and wherein the second polypeptide does not comprise a TGF β superfamily type I or type II receptor polypeptide; wherein the first member of the interaction pair comprises an Fc domain, and wherein the second member of the interaction pair comprises an Fc domain.

According to a second aspect, the present invention provides a pharmaceutical preparation comprising the protein complex of the invention and a pharmaceutically acceptable carrier.

According to a third aspect, the present invention provides a method for treating a patient having a TGF β superfamily-associated condition, comprising administering to the patient the protein complex of the invention or the pharmaceutical preparation of the invention.

According to a fourth aspect, the present invention provides the use of a protein complex of the invention or a pharmaceutical preparation of the invention for the manufacture of a medicament for the treatment of a TGF β superfamily-associated condition in a patient.

In part, the disclosure provides heteromultimeric complexes comprising a single TGF-beta superfamily type I or type II serine/threonine kinase receptor polypeptide (*e.g.*, an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, ActRIIB, TGFBR2, BMPRII, or MISRII polypeptide), including fragments and variants thereof. These constructs may be referred to herein as “single-arm” polypeptide complexes. Optionally, single-arm polypeptide complexes disclosed herein (*e.g.*, a single-arm ActRIIB polypeptide complex, such as an ActRIIB-Fc:Fc heterodimer) have different ligand-binding specificities/profiles compared to a corresponding homodimeric complex (*e.g.*, an ActRIIB homodimer, such as an ActRIIB-Fc:ActRIIB-Fc). Novel properties are exhibited by heteromultimeric polypeptide complexes comprising a single domain of a TGF-beta superfamily type I or type II serine/threonine kinase receptor polypeptide, as shown by Examples herein.

Heteromultimeric structures include, for example, heterodimers, heterotrimers, and higher order complexes. 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, TGFBR2, BMPRII, and MISRII, as well as truncations and variants thereof, or 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, heteromultimeric complexes 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- β 1, TGF- β 2, TGF- β 3, 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 less than or equal to 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} , or 10^{-12} . In general, heteromultimer complexes 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, protein complexes 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, protein
5 complexes 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).

In certain aspects, protein complexes 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 or type II
10 receptor polypeptide and the amino acid sequence of a first member of an interaction pair and the second polypeptide comprises a second member of the interaction pair and does not contain an amino acid sequence of a TGF-beta superfamily type I or type II receptor polypeptide. Optionally, the second polypeptide comprises, in addition to the second member of the interaction pair, a further polypeptide sequence that is not a TGF-beta
15 superfamily type I or type II receptor polypeptide and may optionally comprise not more than 5, 10, 15, 20, 30, 40, 50, 100, 200, 300, 400 or 500 amino acids. Optionally, the TGF-beta superfamily type I or type II 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 or type II receptor polypeptide
20 and the amino acid sequence of the first member of the interaction pair. Examples of linkers include, but are not limited to, the sequences TGGG, TGGGG, SGGGG, GGGGS, and GGG.

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
25 although operative embodiments may also employ an interaction pair that forms a homodimeric complex. 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
30 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.

5 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. 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. Optionally, a first member of an interaction pair may comprise an amino acid sequence that is derived from an Fc domain of an IgG1, IgG2, IgG3, or IgG4 immunoglobulin. For example, the first member of an interaction pair may comprise, consist essentially of, or consist of an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to any one of SEQ ID NOs: 200-214. Optionally, a second member of an interaction pair may
10 comprise an amino acid sequence that is derived from an Fc domain of an IgG1, IgG2, IgG3, or IgG4. 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 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to any one of SEQ ID NOs: 200-214. In some embodiments, a first member and a second member of an interaction pair
15 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. Optionally, a first member and/or a second member of an interaction pair (*e.g.*, an asymmetric pair or an unguided interaction pair) comprise a modified constant domain of an immunoglobulin,
20 including, for example, a modified Fc portion of an immunoglobulin. For example, protein complexes of the disclosure may comprise a first Fc portion of an IgG comprising an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group: SEQ ID NOs: 200-214 and a second Fc portion of an IgG, which may be the same or different from the amino acid
25 sequence of the first modified Fc portion of the IgG, comprising an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group: SEQ ID NOs: 200-214.
30 In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a single type I or type II TGF-beta superfamily receptor polypeptide, wherein the

TGF-beta superfamily receptor polypeptide is derived from an ActRIIA receptor. For example, ActRIIA polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ActRIIA sequence disclosed herein (*e.g.*, SEQ ID NOs: 9, 10, 11, 101, 103, 401, and 402). Optionally, ActRIIA polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 21-30 (*e.g.*, amino acid residues 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) SEQ ID NO: 9, and b) 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. Optionally, ActRIIA polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to ActRIIA. For example, an ActRIIA polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ActRIIA polypeptide and the heterologous polypeptide (*e.g.*, SEQ ID NOs: 101, 103, 401, and 402). In some embodiments, multimerization domains described herein comprise one component of an interaction pair. Heteromeric complexes that comprise an ActRIIA polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an ActRIIB receptor. For example, ActRIIB polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ActRIIB sequence disclosed herein (*e.g.*, SEQ ID NOs: 1, 2, 3, 4, 5, 6, 104, 106, 403, and 404). Optionally, ActRIIB polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) 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 b) 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. Optionally, ActRIIB polypeptides of the disclosure may be fusion proteins

that further comprise one or more portions (domains) that are heterologous to ActRIIB. For example, an ActRIIB polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ActRIIB polypeptide and the heterologous polypeptide (*e.g.*, SEQ ID NOs: 104, 106, 403, and 404).

5 In some embodiments, multimerization domains described herein comprise one component of an interaction pair. Heteromeric complexes that comprise an ActRIIB polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

10 In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from a TGFBR2 receptor. For example, TGFBR2 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an
15 TGFBR2 sequence disclosed herein (*e.g.*, SEQ ID NOs: 42, 43, 67, 68, 113, 115, 409, and 410). Optionally, TGFBR2 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 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 or 51 of SEQ
20 ID NO: 42, and b) ends at any one of amino acids 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165 or 166 of SEQ ID NO: 42. Optionally, TGFBR2 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 23, 24, 25, 26, 27, 28, 29,
25 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44 of SEQ ID NO: 67, and b) ends at any one of amino acids 163, 164, 165, 166, 167, 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. Optionally, TGFBR2 polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to TGFBR2. For example, a
30 TGFBR2 polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the TGFBR2 polypeptide and the heterologous polypeptide (*e.g.*, SEQ ID NOs: 113, 115, 409, and 410). In some embodiments, multimerization domains described herein comprise one component of

an interaction pair. Heteromeric complexes that comprise a TGFBR2 polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

5 In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from a BMPRII receptor. For example, BMPRII polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a
10 BMPRII sequence disclosed herein (*e.g.*, SEQ ID NOs: 46, 47, 71, 72, 107, 109, 405, and 406). Optionally, BMPRII polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 27-34 (*e.g.*, amino acid residues 27, 28, 29, 30, 31, 32, 33, and 34) SEQ ID NO: 46 or 71, and b) ends at any one of
15 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. Optionally, BMPRII polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to BMPRII. For example, a BMPRII polypeptide may be fused to a heterologous polypeptide
20 that comprises a multimerization domain, optionally with a linker domain positioned between the BMPRII polypeptide and the heterologous polypeptide (*e.g.*, SEQ ID NOs: 107, 109, 405, and 406). Heteromeric complexes that comprise a BMPRII polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor
25 polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an MISRII receptor. For example, MISRII polypeptides may comprise, consist essentially of, or consist of an amino acid
30 sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an MISRII sequence disclosed herein (*e.g.*, SEQ ID NOs: 50, 51, 75, 76, 79, 80, 110, 112, 407, and 408). Optionally, MISRII polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100%

identical to a polypeptide that a) begins at any one of amino acids of 17-24 (e.g., amino acid residues 17, 18, 19, 20, 21, 22, 23, and 24) SEQ ID NO: 50, 75, or 79, and b) 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. Optionally, MISRII polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to MISRII. For example, an MISRII polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the MISRII polypeptide and the heterologous polypeptide (e.g., SEQ ID NOs: 110, 112, 407, and 408). In some embodiments, multimerization domains described herein comprise one component of an interaction pair. Heteromeric complexes that comprise an MISRII polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an ALK1 receptor. For example, ALK1 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ALK1 sequence disclosed herein (e.g., SEQ ID NOs: 14, 15, 116, 118, 411, and 412). Optionally, ALK1 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) 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, and b) 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. Optionally, ALK1 polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to ALK1. For example, an ALK1 polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ALK1 polypeptide and the heterologous polypeptide (e.g., SEQ ID NOs: 116, 118, 411, and 412). Heteromeric complexes that comprise an ALK1 polypeptide do not comprise another type I or type II

TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an ALK2 receptor. For example, ALK2 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ALK2 sequence disclosed herein (*e.g.*, SEQ ID NOs: 18, 19, 119, 121, 413, and 414).

Optionally, ALK2 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 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 b) 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. Optionally, ALK2 polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to ALK2. For example, an ALK2 polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ALK2 polypeptide and the heterologous polypeptide (*e.g.*, SEQ ID NOs: 119, 121, 413, and 414).

Heteromeric complexes that comprise an ALK2 polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an ALK3 receptor. For example, ALK3 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ALK3 sequence disclosed herein (*e.g.*, SEQ ID NOs: 22, 23, 122, 124, 415, and 416).

Optionally, ALK3 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) 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 b) 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. Optionally, ALK3 polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to ALK3. For example, an

5 ALK3 polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ALK3 polypeptide and the heterologous polypeptide (e.g., SEQ ID NOs: 122, 124, 415, and 416). Heteromeric complexes that comprise an ALK3 polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional

10 polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an ALK4 receptor. For example, ALK4 polypeptides may comprise, consist essentially of, or consist of an amino acid

15 sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ALK4 sequence disclosed herein (e.g., SEQ ID NOs: 26, 27, 83, 84, 125, 127, 417, and 418). Optionally, ALK4 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 23-34 (e.g., amino acid residues

20 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34) SEQ ID NO: 26 or 83, and b) 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. Optionally, ALK4 polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to ALK4.

25 For example, an ALK4 polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ALK4 polypeptide and the heterologous polypeptide (e.g., SEQ ID NOs: 125, 127, 417, and 418). Heteromeric complexes that comprise an ALK4 polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional

30 polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an ALK5 receptor. For example,

ALK5 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ALK5 sequence disclosed herein (*e.g.*, SEQ ID NOs: 30, 31, 87, 88, 128, 130, 419, and 420). Optionally, ALK5 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 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 b) 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. Optionally, ALK5 polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to ALK5. For example, an ALK5 polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ALK5 polypeptide and the heterologous polypeptide (*e.g.*, SEQ ID NOs: 128, 130, 419, and 420). Heteromeric complexes that comprise an ALK5 polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an ALK6 receptor. For example, ALK6 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ALK6 sequence disclosed herein (*e.g.*, SEQ ID NOs: 34, 35, 91, 92, 131, 133, 421, and 422). Optionally, ALK6 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 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 b) 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. Optionally, ALK6 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that a) begins at any one of amino acids of 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, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62) SEQ ID NO: 91, and b) 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. Optionally, ALK6 polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to ALK6. For example, an ALK6 polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ALK6 polypeptide and the heterologous polypeptide (e.g., SEQ ID NOs: 131, 133, 421, and 422). Heteromeric complexes that comprise an ALK6 polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I or type II TGF-beta superfamily receptor polypeptide, wherein the TGF-beta superfamily receptor polypeptide is derived from an ALK7 receptor. For example, ALK7 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ALK7 sequence disclosed herein (e.g., SEQ ID NOs: 38, 39, 134, 136, 301, 302, 305, 306, 309, 310, 313, 423, and 424). Optionally, ALK7 polypeptides may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that begins at any one of amino acids 21-28 of SEQ ID NO: 38 (e.g., amino acids 21, 22, 23, 24, 25, 26, 27, or 28) and ends at any one of amino acids 92-113 of SEQ ID NO: 38 (e.g., amino acids 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, or 113 of SEQ ID NO: 38). Optionally, ALK7 polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to ALK7. For example, an ALK7 polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the ALK7 polypeptide and the heterologous polypeptide (e.g., SEQ ID NOs: 134, 136, 423, and 424). Heteromeric complexes that comprise an ALK7 polypeptide do not comprise another type I or type II TGF-beta superfamily receptor polypeptide but may contain additional polypeptides that are not type I or type II TGF-beta superfamily receptor polypeptides.

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, including any fusion proteins comprising members of an interaction pair. Nucleic acids disclosed herein may be operably linked to a promoter for expression, and the disclosure further provides cells transformed with such recombinant polynucleotides. Preferably 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 a polypeptide. 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 a 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.

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.

The disclosure further provides methods for use of the protein complexes and pharmaceutical preparations described herein for the treatment or prevention of various TGF-beta associated conditions, including without limitation diseases and disorders associated

with, for example, cancer, muscle, bone, fat, red blood cells, metabolism, fibrosis and other tissues that are affected by one or more ligands of the TGF-beta superfamily.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows schematic examples of single-arm heteromeric protein complexes comprising either a type I receptor polypeptide or a type II receptor polypeptide. Such complexes 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 2 shows a schematic example of a single-arm 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) or 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). In the illustrated embodiment, the type I or type II receptor polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair (“B”), which associates with a second member of an interaction pair (“C”). In the 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 (B, C) 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.

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).

5 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 a single-arm ActRIIB-Fc:Fc heterodimeric
10 protein complex compared to ActRIIB-Fc homodimer. For each protein complex, ligands are ranked by off-rate (k_{off} or k_d), 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. Ligands of particular interest are highlighted in bold while others are represented in
15 gray, and solid black lines indicate ligands whose binding to heterodimer is enhanced or unchanged compared with homodimer, whereas dashed lines indicate substantially reduced binding compared with homodimer. As shown, ActRIIB-Fc homodimer binds to each of five high affinity ligands with similarly high affinity, whereas single-arm ActRIIB-Fc discriminates more readily among these ligands. Thus, single-arm ActRIIB-Fc binds strongly
20 to activin B and GDF11 and with intermediate strength to GDF8 and activin A. In further contrast to ActRIIB-Fc homodimer, single-arm ActRIIB-Fc displays only weak binding to BMP10 and no binding to BMP9. These data indicate that single-arm ActRIIB-Fc has greater ligand selectivity than homodimeric ActRIIB-Fc.

Figure 7 shows ligand binding data for a single-arm ALK3-Fc:Fc heterodimeric
25 protein complex compared to ALK3-Fc homodimer. Format is the same as for Figure 6. As shown, single-arm ALK3-Fc heterodimer retains the exceptionally tight binding to BMP4 observed with ALK3-Fc homodimer, whereas it exhibits reduced strength of binding to BMP2 and therefore discriminates better between BMP4 and BMP2 than does ALK3-Fc homodimer. Single-arm ALK3-Fc also discriminates better among BMP5 (intermediate
30 binding), GDF7 (weak binding), and GDF6 (no binding) compared to ALK3-Fc homodimer, which binds these three ligands with very similar strength (all intermediate). These data indicate that single-arm ALK3-Fc has greater ligand selectivity than homodimeric ALK3-Fc.

Figure 8 shows ligand binding data for a single-arm ActRIIA-Fc:Fc heterodimeric protein complex compared to ActRIIA-Fc homodimer. Format is the same as for Figure 6. As shown, ActRIIA-Fc homodimer exhibits preferential binding to activin B combined with strong binding to activin A and GDF11, whereas single-arm ActRIIA-Fc has a reversed preference for activin A over activin B combined with greatly enhanced selectivity for activin A over GDF11 (weak binder). These data indicate that single-arm ActRIIA-Fc has substantially different ligand selectivity than homodimeric ActRIIA-Fc.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

In part, the present disclosure relates to single-arm heteromultimer complexes comprising an extracellular domain of a TGF β superfamily type I receptor polypeptide or an extracellular domain of a TGF β superfamily type II receptor polypeptide, methods of making such single-arm heteromultimer complexes, and uses thereof. As described herein, single-arm heteromultimer complexes may comprise an extracellular domain of a TGF β superfamily type I receptor polypeptide selected from: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7, or an extracellular domain of a TGF β superfamily type II receptor polypeptide selected from: ActRIIA, ActRIIB, TGFBR1, BMPRII, and MISRII. In certain preferred embodiments, heteromultimer complexes of the disclosure have an altered profile of binding to TGF β superfamily ligands relative to a corresponding homomultimer complex (*e.g.*, an ActRIIB-Fc:Fc heterodimer compared to an ActRIIB-Fc:ActRIIB-Fc homodimer complex).

The TGF- β superfamily is comprised of over thirty 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 bond. This disulfide bond traverses through a ring
5 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 (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
10 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
15 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
20 evolved branch, which includes, *e.g.*, the TGF-betas, activins, GDF8, GDF9, GDF11, BMP3 and nodal. 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. See, *e.g.* Hinck (2012) *FEBS Letters* 586:1860-1870.

TGF-beta isoforms are the founding members of the TGF-beta superfamily, of which
25 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 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
30 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 occurs in neurons and astroglial cells of the embryonic nervous system. TGF-beta2 is also 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.*, Kubiczko et al., (2012) Journal of Translational Medicine 10:183.

Activins are members of the TGF-beta superfamily that were initially discovered as regulators of follicle-stimulating hormone secretion, but subsequently various reproductive and non-reproductive roles have been characterized. Principal activin forms A, B, and AB 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” 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 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 proteins that bind certain TGF-beta superfamily ligands with high affinity and thereby inhibit ligand activity. Curiously, some of these endogenous antagonists resemble TGF-beta superfamily ligands themselves.

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 gradually 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 *ACVRL2* 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), a 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 position 206 of ALK2 is a 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 *BMPRIA* 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 chondrodysplasia and limb defects in both humans and mice.

- 5 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.
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- 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.
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- Transforming growth factor beta receptor II (TGFBRII), encoded by the *TGFBRII* 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.*,
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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 *BMPRII* gene, is a type II receptor that is known to bind BMP ligands including BMP7 and BMP4. 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 with 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 superfamily 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) *Biochimica Medica* 21(3): 219-30 and OMIM entry 600956.

In certain aspects, the present disclosure relates to the use of single-arm heteromultimer complexes comprising an extracellular domain of a TGFβ superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) or an extracellular domain of a TGFβ superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBR2, BMPRII, and MISRII), preferably soluble heteromultimer complexes, 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.*, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, Nodal, GDF8, GDF11, BMP6 and/or BMP7). As described herein, such antagonist single-arm heteromultimer complexes may be useful for the treatment or prevention of various TGF-beta associated conditions, including without limitation diseases and disorders associated with, for example, cancer, muscle, bone, fat, red blood cells, metabolism, fibrosis and other tissues that are affected by one or more ligands of the TGF-beta superfamily.

In particular, the data of the present disclosure demonstrates that single-arm heteromultimer complexes comprising an extracellular domain of a TGFβ superfamily type I

receptor polypeptide or an extracellular domain of a TGF β superfamily type II receptor polypeptide have different ligand selectivity 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 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 chain and Y represents a second polypeptide chain. Higher-order heteromers and oligomeric structures are designated herein in a corresponding manner. In certain embodiments a heteromultimer is recombinant (e.g., one or more polypeptide component may be a recombinant protein), isolated and/or purified.

“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.

As used herein “does not substantially bind to X ” is intended to mean that an agent has a K_D that is greater than about 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} or greater (*e.g.*, no detectable binding by the assay used to determine the K_D) for “ X ”.

2. Heteromultimer Complexes Comprising Single-Arm TGF β Superfamily Receptor Polypeptides

In certain aspects, the disclosure concerns heteromultimer protein complexes comprising one or more single-arm TGF-beta superfamily type I or type II receptor polypeptides. In certain 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 type I or 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 second member of the interaction pair, and wherein the second polypeptide does not comprise a type I or type II receptor polypeptide. 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. As described herein, one member of the interaction pair may be fused to a type I or type II receptor polypeptide, such as a polypeptide comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequence of any of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 9, 10, 11, 14, 15, 18, 19, 22, 23, 26, 27, 30, 31, 34, 35, 38, 39, 42, 43, 46, 47, 50, 51, 67, 68, 71, 72, 75, 76, 79, 80, 83, 84, 87, 88, 91, 92, 301, 302, 305, 306, 309, 310, and 313. Preferably, the interaction pair is selected to confer an improved serum half-life, or to act as an adapter on to which another moiety, such as a polyethylene glycol moiety, is attached to provide an improved serum half-life relative to the monomeric form of the type I or type II receptor polypeptide.

As shown herein, monomeric (single-arm) forms of TGF-beta superfamily type I or type II receptors can exhibit substantially altered ligand-binding selectivity compared to their corresponding homodimeric forms, but the monomeric forms tend to have a short serum residence time (half-life), which is undesirable in the therapeutic setting. A common mechanism for improving serum half-life is to express a polypeptide as a homodimeric fusion protein with a constant domain portion (e.g., an Fc portion) of an IgG. However, TGF-beta superfamily receptor polypeptides expressed as homodimeric proteins (e.g., in an Fc fusion construct) may not exhibit the same activity profile as the monomeric form. As demonstrated herein, the problem may be solved by fusing the monomeric form to a half-life extending moiety, and surprisingly, this can be readily achieved by expressing such proteins as an asymmetric heterodimeric fusion protein in which one member of an interaction pair is fused to a TGF-beta superfamily receptor polypeptide and another member of the interaction pair is fused to either no moiety or to a heterologous moiety, resulting in a novel ligand-binding profile coupled with an improvement in serum half-life conferred by the interaction pair.

In certain aspects, the present disclosure relates to single-arm heteromultimer complexes comprising at least one TGF-beta superfamily type I receptor polypeptide (*e.g.*,

ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7 as well as SEQ ID NOs: 14, 15, 18, 19, 22, 23, 26, 27, 30, 31, 34, 35, 38, 39, 83, 84, 87, 88, 91, 92, 301, 302, 305, 306, 309, 310, 313) or at least one TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBR2, BMPRII, and MISRII as well SEQ ID NOs: 1, 2, 3, 4, 5, 6, 9, 10, 11, 42, 43, 46, 47, 50, 51, 67, 68, 71, 72, 75, 76, 79, and 80), which are generally referred to herein as “single-arm heteromultimer complexes of the disclosure” or “TGF-beta superfamily receptor single-arm heteromultimer complexes”. Preferably, single-arm heteromultimer complexes of the disclosure are soluble, *e.g.*, a single-arm heteromultimer complex comprises a soluble portion of at least one TGFβ superfamily type I receptor polypeptide or 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 or type II receptor. Therefore, in some embodiments, single-arm heteromultimer complexes 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) or an extracellular domain of a TGFβ superfamily type II receptor polypeptide (*e.g.*, one or more ActRIIA, ActRIIB, TGFBR2, BMPRII, and/or MISRII receptor extracellular domains). Exemplary extracellular domains of ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, ActRIIB, TGFBR2, 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.*, single-arm heteromultimer complexes compositions and uses thereof).

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. Any of the heteromeric complexes described herein may comprise such domain of a type I or type II receptor of the TGF-beta superfamily. The core ligand-binding domains of TGFβ superfamily receptors, as demarcated by the outermost of these conserved cysteines, correspond 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 (ALK 5 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 by 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, 28, 29, 30, 31, 32, 33, 34, 35, 36, or 37 residues 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 other preferred embodiments, single-arm heteromultimer complexes of the disclosure bind to and 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- β 1, TGF- β 2, TGF- β 3, 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, single-arm heteromultimer complexes of the disclosure may be used to antagonize intracellular signaling transduction (*e.g.*, Smad 2/3 and/or Smad 1/5/8 signaling) initiated by one or more TGF β superfamily ligands. As described herein, such antagonist heteromultimer complexes may be for the treatment or prevention of various TGF-beta associated conditions, including without limitation diseases and disorders associated with, for example, cancer, muscle, bone, fat, red blood cells, metabolism, fibrosis and other tissues that are affected by one or more ligands of the TGF-beta superfamily. In some embodiments, single-arm heteromultimer complexes of the disclosure have different ligand-binding profiles in comparison to their corresponding homomultimer complex (*e.g.*, an ActRIIB-Fc:Fc heterodimer vs. a corresponding ActRIIB-Fc:ActRIIB-Fc or Fc:Fc homodimer). As described herein, single-arm heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures based on a single-arm unitary complex. In certain preferred embodiments, single-arm heteromultimer complexes of the disclosure are heterodimers.

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 No. WO 2006/012627, which is incorporated herein by reference in its entirety. Numbering of amino acids for all ActRIIB-related polypeptides described herein is based on the numbering of the human ActRIIB precursor protein sequence provided below (SEQ ID NO: 1), unless specifically designated otherwise.

The human ActRIIB precursor protein sequence is as follows:

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1  MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
51 GEQDKRLHCY ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
20 101 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
25 351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKMRPTI KDHWLKHGGL
451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501 TNVDLPPKES SI (SEQ ID NO: 1)

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

The processed extracellular ActRIIB polypeptide sequence is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDD
 FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPTAPT (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 $\Delta 15$ 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.

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

1 MTAPWVALAL LWGSLCAGSG **RGEAETRECI** **YYNANWELER** **TNQSGLERCE**
 51 **GEQDKRLHCY** **ASWANSSGTI** **ELVKKGCWLD** **DFNCYDRQEC** **VATEENPQVY**
 101 **FCCCEGNFCN** **ERFTHLPEAG** **GPEVTYEPPT** **TAPTLLTVLA** YSLLPIGGLS
 151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
 201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
 251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNECHV AETMSRGLSY
 301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
 351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
 401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKMRPTI 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**.

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

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDD
 FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVITYEPPPTAPT (SEQ ID
 NO: 5)

In some embodiments, the protein may be produced with an “SGR...” sequence at the
 5 N-terminus. The C-terminal “tail” of the extracellular domain is indicated by single
 underline. The sequence with the “tail” deleted (a $\Delta 15$ sequence) is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDD
 FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO: 6)

A nucleic acid sequence encoding the human ActRIIB precursor protein is shown
 10 below (SEQ ID NO: 7), consisting of nucleotides 25-1560 of Genbank Reference Sequence
 NM_001106.3, which encode amino acids 1-513 of the ActRIIB precursor. The sequence as
 shown provides an arginine at position 64 and may be modified to provide an alanine instead.
 The signal sequence is underlined.

1 ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGGAT CGCTGTGCGC
 15 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
 20 301 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
 25 551 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
 30 801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951 TGCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA

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1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101 TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
5 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 TCGCTTGTC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
10 1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC
1501 ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC (SEQ ID NO: 7)

```

A nucleic acid sequence encoding processed extracellular human ActRIIB polypeptide is as follows (SEQ ID NO: 8). The sequence as shown provides an arginine at position 64, and may be modified to provide an alanine instead.

```

15      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
20 251 GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
      301 GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
(SEQ ID NO: 8)

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An alignment of the amino acid sequences of human ActRIIB soluble extracellular domain and human ActRIIA soluble extracellular domain are illustrated in Figure 3. This alignment indicates amino acid residues within both receptors that are believed to directly contact ActRII ligands. Figure 4 depicts a multiple-sequence alignment of various vertebrate ActRIIB proteins and human ActRIIA. From these alignments is it possible to predict key amino acid positions within the ligand-binding domain that are important for normal ActRII-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant to substitution without significantly altering normal ActRII-ligand binding activities. ActRII proteins have been characterized in the art in terms of structural and functional characteristics, particularly with respect to ligand binding. See, *e.g.*, 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.

For example, 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 and ActRIIB-based GDF traps ending at 128 (with respect to SEQ ID NO: 1) or later should retain ligand-binding activity. ActRIIB polypeptides and ActRIIB-based GDF traps 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. See, *e.g.*, 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 and ActRIIB-based GDF traps beginning at position 20, 21, 22, 23, and 24 (with respect to

SEQ ID NO: 1) should retain general ligand-binding activity, and ActRIIB polypeptides and ActRIIB-based GDF traps beginning at positions 25, 26, 27, 28, and 29 (with respect to SEQ ID NO: 1) are also expected to retain ligand-binding activity. Data shown in, *e.g.*, U.S. Patent No. 7,842,663 demonstrates that, surprisingly, an ActRIIB construct beginning at 22, 23, 24, or 25 will have the most activity.

Taken together, an active portion (*e.g.*, ligand-binding portion) of ActRIIB comprises amino acids 29-109 of SEQ ID NO: 1. Therefore ActRIIB polypeptides of the present disclosure may, for example, comprise an amino acid sequence that is at least 80%, 85%, 90%, 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 amino acid 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 amino acid 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.*, position 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) or 21-29 (*e.g.*, position 21, 22, 23, 24, 25, 26, 27, 28, or 29) and end at a position from 119-134 (*e.g.*, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-133 (*e.g.*, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133), 129-134 (*e.g.*, 129, 130, 131, 132, 133, or 134), or 129-133 (*e.g.*, 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.*, 20, 21, 22, 23, or 24), 21-24 (*e.g.*, 21, 22, 23, or 24), or 22-25 (*e.g.*, 22, 23, or 25) and end at a position from 109-134 (*e.g.*, 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.*, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) or 129-134 (*e.g.*, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Variants within these ranges are also contemplated, particularly those having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 1.

The disclosure includes the results of an analysis of composite ActRIIB structures, shown in Figure 3, demonstrating that the 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. R40 is a K in *Xenopus*, indicating that basic amino acids at this position will be tolerated. Q53 is R in bovine ActRIIB and K in

Xenopus ActRIIB, and therefore amino acids including R, K, Q, N and H will be tolerated at this position. Thus, a general formula for an ActRIIB polypeptide of the disclosure is one that comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1, optionally beginning at a position ranging from 20-24 (*e.g.*, 20, 21, 22, 23, or 24) or 22-25 (*e.g.*, 22, 23, 24, or 25) and ending at a position ranging from 129-134 (*e.g.*, 129, 130, 131, 132, 133, or 134), 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. 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. See, *e.g.*, 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. See, *e.g.*, U.S. Patent No. 7,842,663.

ActRIIB is well-conserved across nearly all vertebrates, with large stretches of the extracellular domain conserved completely. Many of the ligands that bind to ActRIIB are also highly conserved. Accordingly, comparisons of ActRIIB sequences from various vertebrate organisms provide insights into residues that may be altered. 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 sequence. The following examples illustrate this approach to defining an active ActRIIB variant. L46 is a valine in Xenopus ActRIIB, 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 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 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 is a Y in Xenopus, and therefore Y or other hydrophobic group, such as I, V or L should

be tolerated. E111 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 is K in *Xenopus*, indicating that basic residues are tolerated at this position, including R and H. A at position 119 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.

The variations described herein may be combined in various ways. 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 64 (basic amino acid), 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, in the ActRIIB polypeptides disclosed herein, the disclosure provides a framework of amino acids that may be conserved. 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 single-arm heteromultimer complexes that comprise at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ActRIIB polypeptides for use in accordance with inventions of the disclosure (*e.g.*, single-arm heteromultimer complexes comprising an ActRIIB polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ActRIIB). In other preferred embodiments, ActRIIB 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, single-arm heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of an amino acid sequence that is at least 80%, 85%, 90%, 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 amino acid 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 amino acid 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, single-arm heteromultimer complexes of the disclosure comprise at least one

ActRIIB polypeptide that comprises, consists, or consists essentially of an amino acid sequence that is at least 80%, 85%, 90%, 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 amino acid 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and

5 ending at a position corresponding to amino acids 109-134 (*e.g.*, ending at amino acid 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, wherein the position corresponding to L79 of SEQ ID NO: 1 is an acidic amino acid (*i.e.*, a D or E amino acid residue). In certain preferred embodiments, single-arm heteromultimer complexes of the disclosure comprise at

10 least one ActRIIB polypeptide that comprises, consists, or consists essentially of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 29-109 of SEQ ID NO: 1. In other preferred embodiments, single-arm heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of an amino acid sequence that is at least 80%,

15 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 29-109 of SEQ ID NO: 1, wherein the position corresponding to L79 of SEQ ID NO: 1 is an acidic amino acid (*i.e.*, a D or E amino acid residue). In some embodiments, single-arm heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of any

20 one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 104, 106, 403, or 404. In some embodiments, single-arm heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 104, 106, 403, or 404, wherein the position corresponding to L79 of SEQ ID NO: 1 is an acidic amino acid (*i.e.*, a D or E amino

25 acid residue). In some embodiments, single-arm heteromultimer complexes of the disclosure comprise, consist, or consist essentially of at least one ActRIIB polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 104, 106, 403, or 404. In some embodiments, single-arm heteromultimer complexes of the disclosure comprise, consist, or consist

30 essentially of at least one ActRIIB polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 104, 106, 403, or 404, wherein the position corresponding to L79 of SEQ ID NO: 1 is an acidic amino acid (*i.e.*, a D or E amino acid residue).

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. Numbering of amino acids for all ActRIIA-related polypeptides described herein is based on the numbering of the human ActRIIA precursor protein sequence provided below (SEQ ID NO: 9), unless specifically designated otherwise.

The human ActRIIA precursor protein sequence is as follows:

```

1  MGAAAKLAFA VFLISCSSGA ILGRSETQEC LFFNANWEKD RTNQTGVEPC
20  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 ANVSWNELC HIAETMARGL
25  301 AYLHEDIPGL KDGHKPAISH RDIKSKNVLL KNNLTACIAD FGLALKFEAG
351 KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR
401 CTAADGPVDE YMLPFEEEIG QHPSLEDMQE VVHKKKRPV LRDYWQKHAG
451 MAMLCETIEE CWDHDAEARL SAGCVGERIT QMQRLTNIIT TEDIVTVVTM
501 VTNVDFPPKE SSL (SEQ ID NO: 9)

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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
 INCYDRTDCVEKKDSPEVYFCCCEGNCNEKFSYFPEMEVTQPTSNPVTPKPP (SEQ ID
 NO: 10)

The C-terminal “tail” of the extracellular domain is indicated by a single underline.

- 5 The sequence with the “tail” deleted (a $\Delta 15$ sequence) is as follows:

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDD
 INCYDRTDCVEKKDSPEVYFCCCEGNCNEKFSYFPEM (SEQ ID NO: 11)

A nucleic acid sequence encoding the human ActRIIA precursor protein is shown
 below (SEQ ID NO: 12), corresponding to nucleotides 159-1700 of Genbank Reference

- 10 Sequence NM_001616.4. The signal sequence is underlined.

	1	<u>ATGGGAGCTG</u>	CTGCAAAGTT	GGCGTTTGCC	GTCTTTCTTA	TCTCCTGTTC
	51	<u>TTCAGGTGCT</u>	ATACTTG GTA	GATCAGAAAC	TCAGGAGTGT	CTTTTCTTTA
	101	ATGCTAATTG	GGAAAAAGAC	AGAACCAATC	AAACTGGTGT	TGAACCGTGT
	151	TATGGTGACA	AAGATAAACG	GCGGCATTGT	TTTGCTACCT	GGAAGAATAT
15	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
20	451	GCGGGGATTG	TCATTTGTGC	ATTTTGGGTG	TACAGGCATC	ACAAGATGGC
	501	CTACCCTCCT	GTA CTTGTTC	CAACTCAAGA	CCCAGGACCA	CCCCCACCTT
	551	CTCCATTACT	AGGTTTGAAA	CCACTGCAGT	TATTAGAAGT	GAAAGCAAGG
	601	GGAAGATTTG	GTTGTGTCTG	GAAAGCCCAG	TTGCTTAACG	AATATGTGGC
	651	TGTCAAAATA	TTTCCAATAC	AGGACAAACA	GTCATGGCAA	AATGAATACG
25	701	AAGTCTACAG	TTTGCCTGGA	ATGAAGCATG	AGAACATATT	ACAGTTCATT
	751	GGTGCAGAAA	AACGAGGCAC	CAGTGTTGAT	GTGGATCTTT	GGCTGATCAC
	801	AGCATTTTCAT	GAAAAGGGTT	CACTATCAGA	CTTTCTTAAG	GCTAATGTGG
	851	TCTCTTGGA	TGAACTGTGT	CATATTGCAG	AAACCATGGC	TAGAGGATTG
	901	GCATATTTAC	ATGAGGATAT	ACCTGGCCTA	AAAGATGGCC	ACAAACCTGC
30	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 CATTGAGGA
 1251 GGAAATTGGC CAGCATCCAT CTCTTGAAGA CATGCAGGAA GTTGTGTGTC
 1301 ATAAAAA GAGGCCTGTT TTAAGAGATT ATTGGCAGAA ACATGCTGGA
 5 1351 ATGGCAATGC TCTGTGAAAC CATTGAAGAA TGTGTTGGGATC ACGACGCAGA
 1401 AGCCAGGTTA TCAGCTGGAT GTGTAGGTGA AAGAATTACC CAGATGCAGA
 1451 GACTAACAAA TATTATTACC ACAGAGGACA TTGTAACAGT GGTCAACAATG
 1501 GTGACAAATG TTGACTTTCC TCCCAAAGAA TCTAGTCTA
 (SEQ ID NO: 12)

10 The nucleic acid sequence encoding processed extracellular ActRIIA polypeptide is as follows:

1 ATACTTGGTA GATCAGAAAC TCAGGAGTGT CTTTCTTTA ATGCTAATTG
 51 GGAAAAAGAC AGAACCAATC AAAGTGGTGT TGAACCGTGT TATGGTGACA
 101 AAGATAAACG GCGGCATTGT TTTGCTACCT GGAAGAATAT TTCTGGTTCC
 15 151 ATTGAAATAG TGAAACAAGG TTGTTGGCTG GATGATATCA ACTGCTATGA
 201 CAGGACTGAT TGTGTAGAAA AAAAGACAG CCCTGAAGTA TATTTTGT
 251 GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATTT TCCGGAGATG
 301 GAAGTCACAC AGCCCACTTC AAATCCAGTT ACACCTAAGC CACCC
 (SEQ ID NO: 13)

20 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
 25 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,
 30 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 single-arm heteromultimer complexes 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.*, single-arm heteromultimer complexes 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, single-arm heteromultimer complexes of the disclosure comprise at least one ActRIIA polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 101, 103, 401, or 402. In some embodiments, single-arm heteromultimer complexes of the disclosure comprise, consist, or consist essentially of at least one ActRIIA polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 101, 103, 401, or 402.

In certain aspects, the present disclosure relates to protein complexes that comprise a TGFBR2 polypeptide. As used herein, the term “TGFBR2” refers to a family of transforming growth factor-beta receptor II (TGFBR2) proteins from any species and variants derived from such proteins by mutagenesis or other modification. Reference to TGFBR2 herein is understood to be a reference to any one of the currently identified forms. Members of the TGFBR2 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 “TGFBR2 polypeptide” includes polypeptides comprising any naturally occurring polypeptide of a TGFBR2 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Numbering of amino acids for all TGFBR2-related polypeptides described herein is based on the numbering of the human TGFBR2 precursor protein sequence below (SEQ ID NO: 42), unless specifically designated otherwise.

The canonical human TGFBR2 precursor protein sequence (NCBI Ref Seq NP_003233.4) is as follows:

1 MGRGLLRGLW PLHIVLWTRI AS**T**IPPHVQK SVNNDMIVTD NNGAVKFPQL

51 CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
 101 CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS
 151 EEEYNTSNPDL LLVIFQVTGI SLLPPLGVAI SVIIIFYCYR VNRQQKLSST
 201 WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTL LLPIELDTLV
 5 251 GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
 301 HENILQFLTA EERKTELKGQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL
 351 GSSLARGIAH LHS DHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
 401 SLRLDPTLSV DDLANSQVVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM
 451 ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI
 10 501 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.

The processed extracellular TGFBR2 polypeptide sequence is as follows:

15 TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV
 VAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDN
 IIFSEEYNTSNPDL LLVIFQ (SEQ ID NO: 43)

20 The nucleic acid sequence encoding TGFBR2 precursor protein is shown below
 (SEQ ID NO:44), corresponding to nucleotides 383-2083 of Genbank Reference Sequence
 NM_003242.5. The signal sequence is underlined.

ATGGGTCGGGGGCTGCTCAGGGGCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGC
CAGCACGATCCCACCGCACGTT CAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACA
 25 ACGGTGCAGTCAAGTTTCCACA ACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGAC
 AACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGT
 CTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACC
 CCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAG
 GAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCTGTAGCTCTGATGAGTGCAATGA
 30 CAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTT
 AAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGTCATCATCATCTTC
 TACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAACCGGCAAGACGCG
 GAAGCTCATGGAGTT CAGCGAGCACTGTGCCATCATCCTGGAAGATGACCGCTCTGACATCA
 GCTCCACGTGTGCCAACACATCAACCACAACACAGAGCTGCTGCCCATTGAGCTGGACACC

CTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGCAGAACACTTCAGA
 GCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCCTCTTGGAAGACAG
 AGAAGGACATCTTCTCAGACATCAATCTGAAGCATGAGAACATACTCCAGTTCCTGACGGCT
 GAGGAGCGGAAGACGGAGTTGGGGAAACAATACTGGCTGATCACCGCCTTCCACGCCAAGGG
 5 CAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGACCTGCGCAAGCTGGGCA
 GCTCCCTCGCCCGGGGATTGCTCACCTCCACAGTGATCACACTCCATGTGGGAGGCCCAAG
 ATGCCCATCGTGACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGAACGACCTAACCTG
 CTGCCTGTGTGACTTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCTGTGGATGACCTGG
 CTAACAGTGGGCAGGTGGGAACTGCAAGATACATGGCTCCAGAAGTCCTAGAATCCAGGATG
 10 AATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACTCCATGGCTCTGGTGCTCTG
 GGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAGATTATGAGCCTCCATTTGGTT
 CCAAGGTGCGGGAGCACCCCTGTGTGCAAAGCATGAAGGACAACGTGTTGAGAGATCGAGGG
 CGACCAGAAATTCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGGTGTGTGAGACGTT
 GACTGAGTGCTGGGACCACGACCCAGAGGCCCGTCTCACAGCCCAGTGTGTGGCAGAACGCT
 15 TCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGCTGCTCGGAGGAGAAGATTCCT
 GAAGACGGCTCCCTAAACACTACCAAA (SEQ ID NO: 44)

The nucleic acid sequence encoding processed extracellular TGFBRII polypeptide is as follows:

ACGATCCCACCGCACGTTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACAACGG
 20 TGCAGTCAAGTTTCCACAACCTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACC
 AGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGTCTGT
 GTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACCCCCAA
 GCTCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAA
 AAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCTGTAGCTCTGATGAGTGCAATGACAAC
 25 ATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAA
 (SEQ ID NO: 45)

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

1 MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT
 30 51 AHPLRHINND MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS
 101 ICEKPQEV CV AVWRKNDENI TLETVCHDPK LPYHDFIED AASPKCIMKE
 151 KKKPGETFFM CSCSSDECND NIIFSEEYNT SNPDLLLVI F QVTGISLLPP
 201 LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH CAILEDERS

251 DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA
 301 VKIFPYEEYA SWKTEKDIFS DINLKHENIL QFLTAEERKT ELGKQYWLIT
 351 AFHAKGNLQE YLTRHVISWE DLRKLGSSLA RGIAHLHSDH TPCGRPKMPI
 401 VHRDLKSSNI LVKNDLTCCL CDFGLSLRLD PTLSDVDDL AN SGQVGTARYM
 5 451 APEVLESRMN LENVESFKQT DVYSMALVLW EMTSRCNAVG EVKDYEPFPG
 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**.

The processed extracellular TGFBR2 polypeptide sequence (isoform A) is as follows:

TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFS
 TCDNQKSCMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAPKC
 IMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLLVIQ (SEQ ID NO: 68)

A nucleic acid sequence encoding the TGFBR2 precursor protein (isoform A) is shown below (SEQ ID NO: 69), corresponding to nucleotides 383-2158 of Genbank Reference Sequence NM_001024847.2. The signal sequence is underlined.

20 ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGC
CAGC**ACGATCCCACCGCACGTT CAGAAGTCGGATGTGGAAATGGAGGCCAGAAAGATGAAA**
TCATCTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATA
GTCACTGACAACAACGGTGCAGTCAAGTTTCCACAACCTGTGTAAATTTTGTGATGTGAGATT
TTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGA
 25 **AGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACA**
GTTTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAA
GTGCATTATGAAGGAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCCTGTAGCTCTG
ATGAGTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTG
CTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGT
 30 **CATCATCATCTTCTACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAA**
CCGGCAAGACGCGGAAGCTCATGGAGTTCAGCGAGCACTGTGCCATCATCCTGGAAGATGAC
CGCTCTGACATCAGCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCCAT
TGAGCTGGACACCCTGGTGGGGAAAGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGC
AGAACACTTCAGAGCAGTTTGAGACAGTGGCAGTCAAGATCTTCCCTATGAGGAGTATGCC

TCTTGGAAGACAGAGAAGGACATCTTCTCAGACATCAATCTGAAGCATGAGAACATACTCCA
 GTTCCTGACGGCTGAGGAGCGGAAGACGGAGTTGGGGAAACAATACTGGCTGATCACCGCCT
 TCCACGCCAAGGGCAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGACCTG
 CGCAAGCTGGGCAGCTCCCTCGCCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATG
 5 TGGGAGGCCCCAAGATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGA
 ACGACCTAACCTGCTGCCTGTGTGACTTTGGGCTTTCCTGCGTCTGGACCCTACTCTGTCT
 GTGGATGACCTGGCTAACAGTGGGCAGGTGGGAACTGCAAGATACATGGCTCCAGAAGTCCT
 AGAATCCAGGATGAATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACTCCATGG
 CTCTGGTGCTCTGGGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAG
 10 CCTCCATTTGGTTCCAAGGTGCGGGAGCACCCCTGTGTGCGAAAGCATGAAGGACAACGTGTT
 GAGAGATCGAGGGCGACCAGAAATTCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGG
 TGTGTGAGACGTTGACTGAGTGCTGGGACCACGACCCAGAGGCCCGTCTCACAGCCCAGTGT
 GTGGCAGAACGCTTCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGCTGCTCGGA
 GGAGAAGATTCTGAAGACGGCTCCCTAAACACTACCAA (SEQ ID NO: 69)

15 A nucleic acid sequence encoding the processed extracellular TGFBRII polypeptide
 (isoform A) is as follows:

ACGATCCCACCGCACGTTTCAGAAGTCGGATGTGGAAATGGAGGCCAGAAAGATGAAATCAT
 CTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATAGTCA
 CTGACAACAACGGTGCAGTCAAGTTTCCACAACCTGTGTAAATTTTGTGATGTGAGATTTTCC
 20 ACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCC
 ACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTT
 GCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGC
 ATTATGAAGGAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCTCTGAGCTCTGATGA
 GTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAG
 25 TCATATTTCAA (SEQ ID NO: 70) .

Either of the foregoing TGF β RII 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)
 30 located near the C-terminus of the TGF β RII ECD, as occurs naturally in the TGF β RII
 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 single-arm heteromultimer complexes that comprise at least one TGFBR2 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, TGFBR2 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, single-arm heteromultimer complexes comprising a TGFBR2 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of TGFBR2). In other preferred embodiments, TGFBR2 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, single-arm heteromultimer complexes of the disclosure comprise at least one TGFBR2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NOs: 42, 43, 67, 68, 113, 115, 409, or 410. In some embodiments, single-arm heteromultimer complexes of the disclosure comprise at least one TGFBR2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to any of the amino acid sequences of SEQ ID NOs: 42, 43, 67, 68, 113, 115, 409, or 410, into which is inserted SEQ ID NO: 95 between the paired glutamate residues as described above. In some embodiments, single-arm heteromultimer complexes of the disclosure consist or consist essentially of at least one TGFBR2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NOs: 42, 43, 67, 68, 113, 115, 409, or 410.

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. Numbering of amino acids for all BMPRII-related polypeptides described herein is based on the numbering of the human BMPRII precursor protein sequence below (SEQ ID NO: 46), unless specifically designated otherwise.

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

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1  MTSSLQRPWR VPWLPWTILL VSTAAASQNQ ERLCAFKDPY QQDLGIGESR
51 ISHENGTILC SKGSTCYGLW EKSKGDINLV KQGCWSHIGD PQECHYEECV
5  101 VTTTPPSIQN GTYRFCCCST DLCNVNFTEN FPPPDTTPLS PPHSFNRDET
151 IIALASVSV LAVLIVALCF GYRMLTGDRK QGLHSMNMME AAASEPSLDL
201 DNLKLLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNF INEKNIYRVP
251 LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS
301 SCRLAHSVTR GLAYLHTELP RGDHYKPAIS HRDLNSRNVL VKNDGTCVIS
10 351 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
15 601 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
20 851 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)

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

The processed extracellular BMPRII polypeptide sequence is as follows:

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SQNQERLCAFKDPYQQDLGIGESRISHENGILCSKGSTCYGLWEKSKGDINLVKQGCWSHI
GDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPD
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30 (SEQ ID NO: 47)

A nucleic acid sequence encoding BMPRII precursor protein is shown below (SEQ ID NO: 48), as follows nucleotides 1149-4262 of Genbank Reference Sequence NM_001204.6. The signal sequence is underlined.

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGT
 5 CAGCACTGCGGCTGCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGC
 AAGACCTTGGGATAGGTGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAA
 GGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAGGGGACATAAATCTTGTAACAACAGG
 ATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCA
 CTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAAT
 10 GTCAACTTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATT
 TAACCGAGATGAGACAATAATCATTGCTTTGGCATCAGTCTCTGTATTAGCTGTTTTGATAG
 TTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAAACAAGGTCTTCACAGTATG
 AACATGATGGAGGCAGCAGCATCCGAACCTCTCTTGATCTAGATAATCTGAAACTGTTGGA
 GCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTG
 15 CTGTAAAAGTGTTTTCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGA
 GTGCCTTTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGC
 AGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATCCCAATGGATCTTTATGCAAGT
 ATTTAAGTCTCCACACAAGTGACTGGGTAAGCTCTTGCCGCTCTTGCTCATTCTGTTACTAGA
 GGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCA
 20 TCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACT
 TTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCA
 GCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAA
 CTTGAGGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGACTAATCTATT
 GGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATG
 25 GCTTTTCAGACAGAGGTTGGAAACCATCCCACCTTTTGAGGATATGCAGGTTCTCGTGTCTAG
 GGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCAC
 TCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGT
 GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
 AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGCAACCTGTCACATAATAGGCGTGTGC
 30 CAAAAATTGGTCCTTATCCAGATTATTCTTCCTCCTCATACATTGAAGACTCTATCCATCAT
 ACTGACAGCATCGTGAAGAATATTTCTCTGAGCATTCTATGTCCAGCACACCTTTGACTAT
 AGGGGAAAAAAACCGAAATTCAATTAACCTATGAACGACAGCAAGCACAAAGCTCGAATCCCCA
 GCCCTGAAACAAGTGTCAACAGCCTCTCCACCAACACAACAACCACAAACACCACAGGACTC
 ACGCCAAGTACTGGCATGACTACTATATCTGAGATGCCATACCCAGATGAAACAAATCTGCA

TACCACAAATGTTGCACAGTCAATTGGGCCAACCCCTGTCTGCTTACAGCTGACAGAAGAAG
 ACTTGGAACCAACAAGCTAGACCCAAAAGAAGTTGATAAGAACCTCAAGGAAAGCTCTGAT
 GAGAATCTCATGGAGCACTCTCTTAAACAGTTCAGTGGCCCAGACCCACTGAGCAGTACTAG
 TTCTAGCTTGCTTTACCCACTCATAAACTTGCAGTAGAAGCAACTGGACAGCAGGACTTCA
 5 CACAGACTGCAAATGGCCAAGCATGTTTGATTCCCTGATGTTCTGCCTACTCAGATCTATCCT
 CTCCCCAAGCAGCAGAACCTTCCCAAGAGACCTACTAGTTTGCCTTTGAACACCAAAAATTC
 AACAAAAGAGCCCCGGCTAAAATTTGGCAGCAAGCACAAATCAAACCTTGAAACAAGTCGAAA
 CTGGAGTTGCCAAGATGAATACAATCAATGCAGCAGAACCTCATGTGGTGACAGTCACCATG
 AATGGTGTGGCAGGTAGAAACCACAGTGTTAACTCCCATGCTGCCACAACCCAATATGCCAA
 10 TGGGACAGTACTATCTGGCCAAACAACCAACATAGTGACACATAGGGCCCAAGAAATGTTGC
 AGAATCAGTTTATTGGTGAGGACACCCGGCTGAATATTAATTCCAGTCCTGATGAGCATGAG
 CCTTTACTGAGACGAGAGCAACAAGCTGGCCATGATGAAGGTGTTCTGGATCGTCTTGTGGA
 CAGGAGGGAACGGCCACTAGAAGGTGGCCGAACATAATTCCAATAACAACAACAGCAATCCAT
 GTTCAGAACAAGATGTTCTTGCACAGGGTGTTCCAAGCACAGCAGCAGATCCTGGGCCATCA
 15 AAGCCCAGAAGAGCACAGAGGCCTAATTCTCTGGATCTTTCAGCCACAAATGTCCTGGATGG
 CAGCAGTATACAGATAGGTGAGTCAACACAAGATGGCAAATCAGGATCAGGTGAAAAGATCA
 AGAAACGTGTGAAAACCTCCCTATTCTCTTAAGCGGTGGCGCCCCCTCCACCTGGGTTCATCTCC
 ACTGAATCGCTGGACTGTGAAGTCAACAATAATGGCAGTAACAGGGCAGTTCATTCCAAATC
 CAGCACTGCTGTTTACCTTGCAGAAGGAGGCACTGCTACAACCATGGTGTCTAAAGATATAG
 20 GAATGAACTGTCTG (SEQ ID NO: 48)

The nucleic acid sequence encoding the extracellular BMPRII polypeptide is as follows:

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGG
 25 TGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATG
 GCCTTTGGGAGAAATCAAAGGGGACATAAATCTTGTAACAAGGATGTTGGTCTCACATT
 GGAGATCCCCAAGAGTGTCATATGAAGAATGTGTAGTAACCTACCACTCCTCCCTCAATTCA
 GAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGAGA
 ATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
 30 (SEQ ID NO: 49)

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

1 MTSSLQRPWR VPWLPWTILL VSTAAASQNQ ERLCAFKDPY QQDLGIGESR
 35 51 ISHENGILC SKGSTCYGLW ESKSGDINLV KQGCWSHIGD PQECHYEECV

101 **VTTTPPSIQN** **GTYRFCCCST** **DLCNVNFTEN** **FPPD TTPLS** **PPHSFNRDET**
 151 IIALASVSV LAVLIVALCF GYRMLTGDRK QGLHSMNMME AAASEPSLDL
 201 DNLKLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNF INEKNIYRVP
 251 LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS
 5 301 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 MSTAMQNERR (SEQ ID NO: 71)

10

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

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

15 SQNQERLCAFKDPYQQDLGIGESRISHENG TILCSKGSTCYGLWEKSKGDINLVKQGCWSHI
 GDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPD TTPLSPPHSFNRDET
 (SEQ ID NO: 72)

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

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGT
CAGCACTGCGGCTGCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGC
 25 AAGACCTTGGGATAGGTGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAA
 GGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAACAACAGG
 ATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCCTATGAAGAATGTGTAGTAACTACCA
 CTCCTCCCTCAATTCAGAATGGAACATAACGTTTCTGCTGTTGTAGCACAGATTTATGTAAT
 GTCAACTTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATT
 30 TAACCGAGATGAGACAATAATCATTGCTTTGGCATCAGTCTCTGTATTAGCTGTTTTGATAG
 TTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAAACAAGGTCTTCACAGTATG
 AACATGATGGAGGCAGCAGCATCCGAACCTCTCTTGATCTAGATAATCTGAAACTGTTGGA
 GCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTG
 CTGTAAAAGTGTTTTCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGA
 35 GTGCCTTTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGC

AGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATCCCAATGGATCTTTATGCAAGT
 ATTTAAGTCTCCACACAAGTGACTGGGTAAGCTCTTGCCGTCTTGCTCATTCTGTTACTAGA
 GGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCA
 TCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACT
 5 TTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCA
 GCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAA
 CTTGAGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGAATAATCTATT
 GGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATG
 GCTTTTCAGACAGAGGTTGGAAACCATCCCACCTTTTGAGGATATGCAGGTTCTCGTGTCTAG
 10 GGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCAC
 TCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGT
 GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
 AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGTAGG (SEQ ID NO: 73)

A nucleic acid sequence encoding an extracellular BMPRII polypeptide (isoform 2)
 15 is as follows:

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGG
 TGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATG
 GCCTTTGGGAGAAATCAAAGGGGACATAAATCTTGTAACAAGGATGTTGGTCTCACATT
 20 GGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCACTCCTCCCTCAATTCA
 GAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGAGA
 ATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
 (SEQ ID NO: 74)

In certain embodiments, the disclosure relates to single-arm heteromultimer
 25 complexes 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.*, single-arm heteromultimer complexes
 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
 30 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, single-arm heteromultimer complexes of the disclosure
 comprise at least one BMPRII 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: 46, 47, 71, 72, 107,

109, 405, or 406. In some embodiments, single-arm 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%, or 99% identical to the amino acid sequence of SEQ ID NO: 46, 47, 71, 72, 107, 109, 405, or 406.

5 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
10 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
15 mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Numbering of amino acids for all MISRII-related polypeptides described herein is based on the numbering of the human MISRII precursor protein sequence below (SEQ ID NO: 50), unless specifically designated otherwise.

The canonical human MISRII precursor protein sequence (NCBI Ref Seq
20 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
25 201 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
30 451 DELWALAVQE RRRPYIPSTW RCFATDPDGL RELLEDCWDA DPEARLTAEC
501 VQQRLAALAH PQESHPFPES CPRGCPPLCP EDCTSIPAPT ILPCRPQRSA
551 CHFSVQQGPC SRNPQPACTL SPV (SEQ ID NO: 50)

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

The processed extracellular MISRII polypeptide sequence is as follows:

PPNRRTC VF FEAPGV RGSTKTLGELLDTGT ELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
 5 RDSDEPGCESLHCDPSPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
 GESIWMAL (SEQ ID NO: 51)

A nucleic acid sequence encoding the MISRII precursor protein is shown below (SEQ ID NO: 52), corresponding to nucleotides 81-1799 of Genbank Reference Sequence NM_020547.2. The signal sequence is underlined.

10 ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
GCGAACCTGTGTGTTCTTTGAGGCCCTGGAGTGC GGGGAAGCACAAAGACACTGGGAGAGC
TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAATGCAAGGATGCCGAGACAGTGA
TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCAGCCCTGGCT
 15 **CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT**
CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
CTGGATGGCACTGGTGCTGCTGGGGCTGTTCTCTCCTCCTGCTGCTGCTGGGCAGCATCA
 TCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGAGCCAGTGCCAGAGCCAAGG
 CCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCA
 20 GGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACCTGGTTG
 CCATCAAGGCCTTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTTGTACGAA
 CTTCCAGGCCTACAGCACGACCACATTGTCCGATTTATCACTGCCAGCCGGGGGGGTCTTG
 CCGCCTGCTCTCTGGGCCCCCTGCTGGTACTGGAAGTGCATCCCAAGGGCTCCCTGTGCCACT
 ACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTGGCCCAG
 25 GGCCTGGCATTCTTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACAGGTATTGCCCA
 CCGAGATCTGAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACC
 TGGGCCTTGCCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCA
 CAAGGCCAGCTGCCATCATGGAAGCTGGCACCCAGAGGTACATGGCACCCAGAGCTCTTGGA
 CAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTACTCTTTGG
 30 CTCTGCTCCTGTGGGAGATACTGAGCCGCTGCCCAGATTTGAGGCCTGACAGCAGTCCACCA
 CCCTTCCAACCTGGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGC
 CTTGGCAGTGCAGGAGAGGAGGCGTCCCTACATCCCATCCACCTGGCGCTGCTTTGCCACAG
 ACCCTGATGGGCTGAGGGAGCTCCTAGAAGACTGTTGGGATGCAGACCCAGAAGCACGGCTG

ACAGCTGAGTGTGTACAGCAGCGCCTGGCTGCCTTGGCCCATCCTCAAGAGAGCCACCCCTT
TCCAGAGAGCTGTCCACGTGGCTGCCCACCTCTCTGCCCAGAAGACTGTACTTCAATTCCTG
CCCCTACCATCCTCCCCTGTAGGCCTCAGCGGAGTGCCTGCCACTTCAGCGTTCAGCAAGGC
CCTTGTTCCAGGAATCCTCAGCCTGCCTGTACCCTTTCTCCTGTG (SEQ ID NO: 52)

5 A nucleic acid sequence encoding the extracellular human MISRII polypeptide is as follows:

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCTGGAGTGCGGGGAAGCACAAAGAC
ACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCC
GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
10 CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC
CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
GCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCA
GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 53)

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

1 MLGSLGLWAL LPTAVEAPPN **RRTC****VF****FEAP** **GVRG****ST****KT****LG** **EL****LD****TG****TE****LP**
051 **RAIR****CL****YS****RC** **CF****GI****WN****LT****QD** **RA****QV****EM****Q****G****CR** **DS****DE****P****G****CE****SL** **HC****DP****SP****RA****HP**
101 **SP****GS****TL****FT****CS** **CG****TD****FC****N****ANY** **SH****L****PP****P****G****S****P****G** **TP****GS****Q****G****P****Q****AA** **PG****ES****I****W****M****A****L****V**
151 **LL****GL****FL****LL****LL** **LL****GS****I****L****L****ALL** **QR****K****NY****R****V****R****GE** **PV****PE****RP****D****SG** **RD****WS****VE****L****Q****EL**
20 201 **PE****LC****FS****Q****V****IR** **EG****GH****AV****V****W****AG** **QL****Q****G****K****L****V****AI****K** **AF****P****P****R****S****V****A****Q****F** **QA****E****R****A****L****Y****E****LP**
251 **GL****Q****H****D****H****I****V****R****F** **IT****AS****R****G****G****P****GR** **LL****S****G****P****LL****V****LE** **LH****P****K****G****S****L****CH****Y** **LT****Q****Y****T****S****D****W****G****S**
301 **SL****R****M****A****L****S****L****A****Q** **GL****A****F****L****H****E****E****R****W** **Q****N****G****Q****Y****K****P****G****I****A** **HR****D****L****S****S****Q****N****V****L** **IR****E****D****G****S****C****A****I****G**
351 **DL****GL****A****L****V****L****P****G** **LT****Q****P****P****A****W****T****P****T** **QP****Q****G****P****A****A****I****M****E** **AG****T****Q****R****Y****M****A****P****E** **LL****D****K****T****L****D****L****Q****D**
401 **WG****M****A****L****R****R****A****D****I** **Y****S****L****A****L****L****L****W****E****I** **LS****R****C****P****D****L****R****P****A** **VH****H****P****S****N****W****P****M****R** **QN****W****A****I****P****L****P****L****M**
25 451 **SY****G****P****W****Q****C****R****R****G** **GV****P****T****S****H****P****P****G****A** **AL****P****Q****T****L****M****G** (SEQ ID NO: 75)

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

The processed extracellular MISRII polypeptide sequence (isoform 2) is as follows:

PPNRRTC VF FEAP GVRG ST KT LG EL LD TG TE LP RAIR CL YS RC CF GI WN LT QD RA QV EM QG C
30 R D S D E P G C E S L H C D P S P R A H P S P G S T L F T C S C G T D F C N A N Y S H L P P P G S P G T P G S Q G P Q A A P
G E S I W M A L (SEQ ID NO: 76)

A nucleic acid sequence encoding the MISRII precursor protein (isoform 2) is shown below (SEQ ID NO: 77), corresponding to nucleotides 81-1514 of Genbank Reference Sequence NM_001164690.1. The signal sequence is underlined.

5 ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
 GCGAACCTGTGTGTTCTTTGAGGCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGC
 TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
 GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGA
 TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGGCT
 CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
 10 CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
 CTGGATGGCACTGGTGCTGCTGGGGCTGTTCTCCTCCTCCTGCTGCTGCTGGGCAGCATCA
 TCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGAGCCAGTGCCAGAGCCAAGG
 CCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCA
 GGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACCTGGTTG
 15 CCATCAAGGCCTTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTTGTACGAA
 CTTCAGGCCTACAGCACGACCACATTGTCCGATTTATCACTGCCAGCCGGGGGGGTCTTG
 CCGCCTGCTCTCTGGGCCCCCTGCTGGTACTGGAAGTGCATCCCAAGGGCTCCCTGTGCCACT
 ACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTGGCCCAG
 GGCCTGGCATTCTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCA
 20 CCGAGATCTGAGCAGCCAGAATGTGCTCATTGCGGAAGATGGATCGTGTGCCATTGGAGACC
 TGGGCCTTGCCCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCA
 CAAGGCCAGCTGCCATCATGGAAGCTGGCACCCAGAGGTACATGGCACCCAGAGCTCTTGGA
 CAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTACTCTTTGG
 CTCTGCTCCTGTGGGAGATACTGAGCCGCTGCCCAGATTTGAGGCCTGCAGTCCACCACCCT
 25 TCCAACCTGGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGCCTTG
 GCAGTGCAGGAGAGGAGGCGTCCCTACATCCCATCCACCTGGCGCTGCTTTGCCACAGACCC
 TGATGGGC (SEQ ID NO: 77)

The nucleic acid sequence encoding processed soluble (extracellular) human MISRII polypeptide (isoform 2) is as follows:

30 CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCTGGAGTGCGGGGAAGCACAAAGAC
 ACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCC
 GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
 CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC

CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
GCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCA
GGTGAGTCCATCTGGATGGCACTG (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 **RRTC**VF**FEAP** **GVRG**ST**KT**LG **ELL**DT**GT**ELP
51 **RAIR**CL**YS**RC **CFGI**WN**LT**QD **RAQV**EM**QG**CR **DSDE**PG**CE**SL **HCDP**SP**RA**HP
101 **SPG**ST**LF**TC **CGT**DFC**N**ANY **SHL**PP**PG**SPG **TPG**S**QG**P**QA**A **PGE**SI**WM**ALV
151 LLGLFLLLLL LLGSIILALL QRKNYRVRGE PVPEPRPD SG RDWSVELQEL
10 201 PELCFSQVIR EGGHAVVWAG QLQGLVAIK AFFPRSV AQF QAERALYELP
251 GLQHDHIVRF ITASRGGPGR LLSGPLLVLE LHPKGS LCHY LTQYTS DWGS
301 SLRMALSLAQ GLAFLHEERW QNGQYKPGIA HRDLSSQ NVL IREDGSCAIG
351 DLGLALVLPG LTQPPAWTPT QPQGPAAIME DPDGLRELL E DCWDADPEAR
401 LTAECVQQRL AALAHPOESH PFPESCPRG C PPLCPEDCT S IPAPTILPCR
15 451 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.

The processed extracellular MISRII polypeptide sequence (isoform 3) is as follows:
PPNRRTC VF FEAPGVRGSTKT LG ELLDTGT ELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
20 RDSDEPGCESLHCDPSPRAHPSPGSTLF TCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
GESIWMAL (SEQ ID NO: 80)

A nucleic acid sequence encoding human MISRII precursor protein (isoform 3) is shown below (SEQ ID NO: 81), corresponding to nucleotides 81-1514 of Genbank Reference Sequence NM_001164691.1. The signal sequence is underlined.

25 ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
GCGAACCTGTGTGTTCTTTGAGGCCCTGGAGTGC GGGGAAGCACAAAGACACTGGGAGAGC
TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGA
TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCAAGCCAGCCCTGGCT
30 CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
CTGGATGGCACTGGTGCTGCTGGGGCTGTTCTCTCTCCTGCTGCTGCTGGGCAGCATCA
TCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGAGCCAGTGCCAGAGCCAAGG

CCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCA
 GGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAAGTGGTTG
 CCATCAAGGCCTTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTTGTACGAA
 CTTCCAGGCCTACAGCACGACCACATTGTCCGATTTATCACTGCCAGCCGGGGGGGTCTGG
 5 CCGCCTGCTCTCTGGGCCCCCTGCTGGTACTGGAAGTGCATCCCAAGGGCTCCCTGTGCCACT
 ACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTGGCCCAG
 GGCCTGGCATTCTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACAGGTATTGCCCA
 CCGAGATCTGAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACC
 TGGGCCTTGCCCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCA
 10 CAAGGCCCAGCTGCCATCATGGAAGACCCTGATGGGCTGAGGGAGCTCCTAGAAGACTGTTG
 GGATGCAGACCCAGAAGCACGGCTGACAGCTGAGTGTGTACAGCAGCGCCTGGCTGCCTTGG
 CCCATCCTCAAGAGAGCCACCCCTTTCCAGAGAGCTGTCCACGTGGCTGCCCACCTCTCTGC
 CCAGAAGACTGTACTTCAATTCCTGCCCCCTACCATCCTCCCCTGTAGGCCTCAGCGGAGTGC
 CTGCCACTTCAGCGTTCAGCAAGGCCCTTGTTCCAGGAATCCTCAGCCTGCCTGTACCCTTT
 15 CTCCTGTG (SEQ ID NO: 81)

A nucleic acid sequence encoding processed soluble (extracellular) human MISRII polypeptide (isoform 3) is as follows:

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCCTGGAGTGCGGGGAAGCACAAAGAC
 ACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCC
 20 GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
 CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC
 CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
 GCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCA
 GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 82)

25 In certain embodiments, the disclosure relates to single-arm heteromultimer complexes 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.*, single-arm heteromultimer complexes comprising a MISRII polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain
 30 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, single-arm heteromultimer complexes of the disclosure

comprise at least one MISRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NOs: 50, 51, 75, 76, 79, 80, 110, 112, 407, or 408. In some embodiments, single-arm heteromultimer complexes of the disclosure consist or consist essentially of at least one MISRII polypeptide that is at least

5 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NOs: 50, 51, 75, 76, 79, 80, 110, 112, 407, or 408.

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

10 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. Numbering of amino acids for all ALK1-related polypeptides described herein is based on the numbering of the human ALK1 precursor protein sequence below (SEQ ID NO: 14), unless

20 specifically designated otherwise.

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

1 MTLGSPRKGL LMLLMALVTQ GDPVKPSRGP LVTCTCESPH CKGPTCRGAW
 51 CTVVLVREEG RHPQEHRCG NLHRELCRGR PTEFVNHYCC DSHLCNHNVS
 25 101 LVLEATQPPS EQPGTDGQLA LILGPVLALL ALVALGVLGL WHVRRRQEKQ
 151 RGLHSELGES SLILKASEQG DSMLGDL LDS DCTTGSGSGL PFLVQRTVAR
 201 QVALVECVGK GRYGEVWRGL WHGESVAVKI FSSRDEQSWF RETEIYNTVL
 251 LRHDNILGFI ASDMTSRNSS TQLWLITHYH EHGSLYDFLQ RQTLEPHLAL
 301 RLAVSAACGL AHLHVEIFGT QGKPAIAHRD FKSRNVLVKS NLQCCIADLG
 30 351 LAVMHSQGSD YLDIGNNPRV GTKRYMAPEV LDEQIRTD CF ESYKWTDIWA
 401 FGLVLWEIAR RTIVNGIVED YRPPFYDVVP NDPSFEDMKK VVCVDQQTPT
 451 IPNRLAADPV LSGLAQMMRE CWYPNPSARL TALRIKKTLO 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**.

The processed extracellular ALK1 polypeptide sequence is as follows:

DPVKPSRGPLVTCTCESPHCKGPTCRGAWCTVVLVREEGRHPQEHRCGNLHRELRCGRPTE
 5 FVNHYCCDSHLNHNVSLEATQPPSEQPGTDGQ (SEQ ID NO: 15)

A nucleic acid sequence encoding human ALK1 precursor protein is shown below (SEQ ID NO: 16), corresponding to nucleotides 284-1792 of Genbank Reference Sequence NM_000020.2. The signal sequence is underlined.

ATGACCTTGGGCTCCCCAGGAAAGGCCTTCTGATGCTGCTGATGGCCTTGGTGACCCAGGG
 10 AGACCCTGTGAAGCCGTCTCGGGGCCGCTGGTGACCTGCACGTGTGAGAGCCCACATTGCA
AGGGGCCCTACCTGCCGGGGGGCCTGGTGACAGTAGTGCTGGTGCGGGAGGAGGGGAGGCAC
CCCCAGGAACATCGGGGCTGCGGGAACCTGCACAGGGAGCTCTGCAGGGGGCGCCCCACCGA
GTTTCGTCAACCACTACTGCTGCGACAGCCACCTCTGCAACCACAACGTGTCCCTGGTGCTGG
AGGCCACCCAACCTCCTTCGGAGCAGCCGGGAACAGATGGCCAGCTGGCCCTGATCCTGGGC
 15 **CCCGTGCTGGCCTTGCTGGCCCTGGTGGCCCTGGGTGTCTTGGGCCTGTGGCATGTCCGACG**
GAGGCAGGAGAAGCAGCGTGGCCTGCACAGCGAGCTGGGAGAGTCCAGTCTCATCCTGAAAG
CATCTGAGCAGGGCGACAGCATGTTGGGGGACCTCCTGGACAGTGACTGCACCACAGGGAGT
GGCTCAGGGCTCCCCTTCCTGGTGACAGGACAGTGGCACGGCAGGTTGCCTTGGTGAGTG
TGTGGGAAAAGGCCGCTATGGCGAAGTGTGGCGGGGCTTGTGGCACGGTGAGAGTGTGGCCG
 20 **TCAAGATCTTCTCCTCGAGGGATGAACAGTCCTGGTTCCGGGAGACTGAGATCTATAACACA**
GTGTTGCTCAGACACGACAACATCCTAGGCTTCATCGCCTCAGACATGACCTCCCGCAACTC
GAGCACGCAGCTGTGGCTCATCACGCACTACCACGAGCACGGCTCCCTCTACGACTTTCTGC
AGAGACAGACGCTGGAGCCCCATCTGGCTCTGAGGCTAGCTGTGTCCGCGGCATGCGGCCTG
GCGCACCTGCACGTGGAGATCTTCGGTACACAGGGCAAACCAGCCATTGCCACCGCGACTT
 25 **CAAGAGCCGCAATGTGCTGGTCAAGAGCAACCTGCAGTGTTCATCGCCGACCTGGGCCTGG**
CTGTGATGCACTCACAGGGCAGCGATTACCTGGACATCGGCAACAACCCGAGAGTGGGCACC
AAGCGGTACATGGCACCCGAGGTGCTGGACGAGCAGATCCGCACGGACTGCTTTGAGTCCTA
CAAGTGGACTGACATCTGGGCCTTTGGCCTGGTGTGTGGGAGATTGCCCGCCGGACCATCG
TGAATGGCATCGTGGAGGACTATAGACCACCCTTCTATGATGTGGTGCCCAATGACCCACGC
 30 **TTTGAGGACATGAAGAAGGTGGTGTGTGTGGATCAGCAGACCCCCACCATCCCTAACCGGCT**
GGCTGCAGACCCGGTCTCTCAGGCCTAGCTCAGATGATGCGGGAGTGCTGGTACCCAAACC
CCTCTGCCCCGACTCACCGCGCTGCGGATCAAGAAGACACTACAAAAAATTAGCAACAGTCCA
GAGAAGCCTAAAGTGATTCAA (SEQ ID NO: 16)

A nucleic acid sequence encoding processed extracellular ALK1 polypeptide is as follows:

GACCCTGTGAAGCCGTCTCGGGGCCCCGCTGGTGACCTGCACGTGTGAGAGCCCCACATTGCAA
 GGGGCCTACCTGCCGGGGGGGCCTGGTGACAGTAGTGCTGGTGCGGGAGGAGGGGAGGCACC
 5 CCCAGGAACATCGGGGCTGCGGGAACCTTGACAGGGAGCTCTGCAGGGGGCGCCCCACCGAG
 TTCGTCAACCACTACTGCTGCGACAGCCACCTCTGCAACCACAACGTGTCCCTGGTGCTGGA
 GGCCACCCAACCTCCTTCGGAGCAGCCGGGAACAGATGGCCAG (SEQ ID NO: 17)

In certain embodiments, the disclosure relates to single-arm heteromultimer complexes that comprise at least one ALK1 polypeptide, which includes fragments,
 10 functional variants, and modified forms thereof. Preferably, ALK1 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, single-arm heteromultimer complexes 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
 15 Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, single-arm heteromultimer complexes of the disclosure comprise at least one ALK1 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: 14, 15, 116, 118, 411, or 412. In some embodiments, single-arm heteromultimer complexes of the disclosure consist or consist
 20 essentially of at least one ALK1 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: 14, 15, 116, 118, 411, or 412.

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-
 25 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
 30 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.

Numbering of amino acids for all ALK2-related polypeptides described herein is based on the numbering of the human ALK2 precursor protein sequence below (SEQ ID NO: 18), unless specifically designated otherwise.

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

1 MVDGVMILPV LIMIALPSPS **MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG**
 51 **QQCFSSLSIN DGFHVYQKGC FQVYEQ GKMT CKTPSPGQA VECCQGDWCN**
 101 **RNITAQLPTK GKSFPQTQNF** HLEVGLIILS VVFAVCLLAC LLGVALRKFK
 10 151 RRNQERLNPR DVEYGTIEGL ITTNVGDSTL ADLLDHSCTS GSGSGLPFLV
 201 QRTVARQITL LECVGKG RYG EVWRGSWQGE NVAVKIFSSR DEKSWFRETE
 251 LYNTVMLRHE NILGFIASDM TSRHSSTQLW LITHYHEMGS LYDYLQLTTL
 301 DTVSCLRIVL SIASGLAHLH IEIFGTQGKP AIAHRDLKSK NILVKKNGQC
 351 CIADLGLAVM HSQSTNQLDV GNNPRVGTKR YMAPEVLDET IQVDCFDSYK
 15 401 RVDIWAFFGLV LWEVARRMVS NGIVEDYKPP FYDVVPNDPS FEDMRKVVCV
 451 DQQRPNIPNR WFS DPTLTSL 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.

- 20 The processed extracellular ALK2 polypeptide sequence is as follows:

MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG **QQCFSSLSIN** DGFHVYQKGC FQVYEQ GKMTCK
 TPPSPGQAVECCQGDWCN **RNITAQLPTK** GKSFPQTQNFHLE (SEQ ID NO: 19)

- 25 A nucleic acid sequence encoding human ALK2 precursor protein is shown below (SEQ ID NO: 20), corresponding to nucleotides 431-1957 of Genbank Reference Sequence NM_001105.4. The signal sequence is underlined.

ATGGTAGATGGAGTGATGATTCTTCCTGTGCTTATCATGATTGCTCTCCCCTCCCCTAGT**AT**
GGAAGATGAGAAGCCCAAGGTCAACCCCAAACTCTACATGTGTGTGTGTGAAGGTCTCTCCT
 GCGGTAATGAGGACCACTGTGAAGGCCAGCAGTGCTTTTCCTCACTGAGCATCAACGATGGC
 TTCCACGTCTACCAGAAAGGCTGCTTCCAGGTTTATGAGCAGGGAAAGATGACCTGTAAGAC
 30 CCCGCCGTCCCCTGGCCAAGCCGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACATCA
 CGGCCCAGCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAGGTT
 GCCTCATTATTCTCTCTGTAGTGTTGCGCAGTATGTCTTTTAGCCTGCCTGCTGGGAGTTGC

TCTCCGAAAATTTAAAAGGCGCAACCAAGAACGCCTCAATCCCCGAGACGTGGAGTATGGCA
 CTATCGAAGGGCTCATCACCACCAATGTTGGAGACAGCACTTTAGCAGATTTATTGGATCAT
 TCGTGTACATCAGGAAGTGGCTCTGGTCTTCCTTTTCTGGTACAAAGAACAGTGGCTCGCCA
 GATTACACTGTTGGAGTGTGTCTGGGAAAGGCAGGTATGGTGAGGTGTGGAGGGGCAGCTGGC
 5 AAGGGGAGAATGTTGCCGTGAAGATCTTCTCCTCCCGTGATGAGAAGTCATGGTTCAGGGAA
 ACGGAATTGTACAACACTGTGATGCTGAGGCATGAAAATATCTTAGGTTTCATTGCTTCAGA
 CATGACATCAAGACACTCCAGTACCCAGCTGTGGTTAATTACACATTATCATGAAATGGGAT
 CGTTGTACGACTATCTTCAGCTTACTACTCTGGATACAGTTAGCTGCCTTCGAATAGTGCTG
 TCCATAGCTAGTGGTCTTGACATTTGCACATAGAGATATTTGGGACCCAAGGGAAACCAGC
 10 CATTGCCCATCGAGATTTAAAGAGCAAAAATATTCTGGTTAAGAAGAATGGACAGTGTGCA
 TAGCAGATTTGGGCCTGGCAGTCATGCATTCCCAGAGCACCAATCAGCTTGATGTGGGGAAC
 AATCCCCGTGTGGGCACCAAGCGCTACATGGCCCCCGAAGTTCTAGATGAAACCATCCAGGT
 GGATTGTTTCGATTCTTATAAAAGGGTCGATATTTGGGCCTTTGGACTTGTTTTGTGGGAAG
 TGGCCAGGCGGATGGTGAGCAATGGTATAGTGAGGATTACAAGCCACCGTTCTACGATGTG
 15 GTTCCCAATGACCCAAGTTTTGAAGATATGAGGAAGGTAGTCTGTGTGGATCAACAAAGGCC
 AAACATACCCAACAGATGGTTCTCAGACCCGACATTAACCTCTCTGGCCAAGCTAATGAAAG
 AATGCTGGTATCAAAATCCATCCGCAAGACTCACAGCACTGCGTATCAAAAAGACTTTGACC
 AAAATTGATAATTCCTTCGACAAATTGAAAACCTGACTGT (SEQ ID NO: 20)

20 A nucleic acid sequence encoding the extracellular ALK2 polypeptide is as follows:

ATGGAAGATGAGAAGCCCAAGGTCAACCCCCAACTCTACATGTGTGTGTGTGAAGGTCTCTC
 CTGCGGTAATGAGGACCACTGTGAAGGCCAGCAGTGCTTTTCCTCACTGAGCATCAACGATG
 GCTTCCACGTCTACCAGAAAGGCTGCTTCCAGGTTTATGAGCAGGGAAAGATGACCTGTAAG
 ACCCCGCCGTCCCCTGGCCAAGCCGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACAT
 25 CACGGCCCAGCTGCCCCTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAG
 (SEQ ID NO: 21)

In certain embodiments, the disclosure relates to single-arm heteromultimer
 complexes that comprise at least one ALK2 polypeptide, which includes fragments,
 30 functional variants, and modified forms thereof. Preferably, ALK2 polypeptides for use in
 accordance with inventions of the disclosure (*e.g.*, single-arm heteromultimer complexes
 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, single-arm heteromultimer complexes of the disclosure comprise at least one ALK2 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: 18, 19, 119, 121, 413, or 414. In some

5 embodiments, single-arm 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%, or 99% identical to the amino acid sequence of SEQ ID NO: 18, 19, 119, 121, 413, or 414.

In certain aspects, the present disclosure relates to protein complexes that comprise an

10 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-

15 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.

20 Numbering of amino acids for all ALK3-related polypeptides described herein is based on the numbering of the human ALK3 precursor protein sequence below (SEQ ID NO: 22), unless specifically designated otherwise.

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

25 1 MPQLYIYIRL LGAYLFIISR VQGQNLDSML HGTGMKSDSD QKKSENGVTL APEDTLPFLK
61 CYCSGHCPDD AINNTCITNG HCFAIEEDD QGETTLASGC MKYEGSDFQC KDSPKAQLRR
121 TIECCRTNLC NQYLQPTLPP VVIGPFFDGS IRWLVLISM AVCIIAMIF SSCFCYKHYC
181 KSISSRRRYN RDLEQDEAFI PVGESLKDLI DQSQSSGSGS GLPLLQVQRTI AKQIQMVRQV
241 GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT VLMRHENILG FIAADIKGTG
30 301 SWTQLYLITD YHENGSLYDF LKCATLDTRA LLKLAYSAC GLCHLHTEIY GTQGKPAIAH
361 RDLKSKNILI KKNSSCCIAD LGLAVKFNSD TNEVDVPLNT RVGTKRYMAP EVLDESLNKN
421 HFQPYIMADI YSFGLIIWEM ARRCITGGIV EEYQLPYINM VPSDPSYEDM REVVCVKRLR
481 PIVSNRWNSD ECLRAVLKLM SECWAHPAS 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.

The processed extracellular ALK3 polypeptide sequence is as follows:

5 1 QNLDSMLHGT GMKSDSDQKK SENGVTLAPE DTLPFLKCYC SGHCPDDAIN NTCITNGHCF
 61 AIIIEEDDQGE TTLASGCMKY EGSDFQCKDS PKAQLRRTIE CCRTNLCNQY LQPTLPPVVI
 121 GPFFDGSIR (SEQ ID NO: 23)

10 A nucleic acid sequence encoding human ALK3 precursor protein is shown below
 (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.

 1 ATGCCTCAGC TATACATTTA CATCAGATTA TTGGGAGCCT ATTTGTTTCAT
 CATTCTCTCGT
 61 GTTCAAGGAC **AGAATCTGGA TAGTATGCTT CATGGCACTG GGATGAAATC**
 15 **AGACTCCGAC**
 121 **CAGAAAAAGT CAGAAAATGG AGTAACCTTA GCACCAGAGG ATACCTTGCC**
 TTTTTTTAAAG
 181 **TGCTATTGCT CAGGGCACTG TCCAGATGAT GCTATTAATA ACACATGCAT**
 AACTAATGGA
 20 241 **CATTGCTTTG CCATCATAGA AGAAGATGAC CAGGGAGAAA CCACATTAGC**
 TTCAGGGTGT
 301 **ATGAAATATG AAGGATCTGA TTTTCAGTGC AAAGATTCTC CAAAAGCCCA**
 GCTACGCCGG
 361 **ACAATAGAAT GTTGTCGGAC CAATTTATGT AACCAGTATT TGCAACCCAC**
 25 **ACTGCCCCCT**
 421 **GTTGTCATAG GTCCGTTTTT TGATGGCAGC ATTCGATGGC TGGTTTTGCT**
 CATTCTATG
 481 **GCTGTCTGCA TAATTGCTAT GATCATCTTC TCCAGCTGCT TTTGTTACAA**
 ACATTATTGC
 30 541 **AAGAGCATCT CAAGCAGACG TCGTTACAAT CGTGATTGG AACAGGATGA**
 AGCATTTATT
 601 **CCAGTTGGAG AATCACTAAA AGACCTTATT GACCAGTCAC AAAGTTCTGG**
 TAGTGGGTCT
 661 **GGACTACCTT TATTGGTTCA GCGAACTATT GCCAAACAGA TTCAGATGGT**
 35 **CCGGCAAGTT**

721 GGTAAGGCC GATATGGAGA AGTATGGATG GGCAAATGGC GTGGCGAAAA
 AGTGGCGGTG
 781 AAAGTATTCT TTACCACTGA AGAAGCCAGC TGGTTTCGAG AAACAGAAAT
 CTACCAAAC
 5 841 GTGCTAATGC GCCATGAAAA CATACTTGGT TTCATAGCGG CAGACATTAA
 AGGTACAGGT
 901 TCCTGGACTC AGCTCTATTT GATTACTGAT TACCATGAAA ATGGATCTCT
 CTATGACTTC
 961 CTGAAATGTG CTACACTGGA CACCAGAGCC CTGCTTAAAT TGGCTTATTC
 10 AGCTGCCTGT
 1021 GGTCTGTGCC ACCTGCACAC AGAAATTTAT GGCACCCAAG GAAAGCCCGC
 AATTGCTCAT
 1081 CGAGACCTAA AGAGCAAAAA CATCCTCATC AAGAAAAATG GGAGTTGCTG
 CATTGCTGAC
 15 1141 CTGGGCCTTG CTGTTAAATT CAACAGTGAC ACAAATGAAG TTGATGTGCC
 CTTGAATACC
 1201 AGGGTGGGCA CCAAACGCTA CATGGCTCCC GAAGTGCTGG ACGAAAGCCT
 GAACAAAAAC
 1261 CACTTCCAGC CCTACATCAT GGCTGACATC TACAGCTTCG GCCTAATCAT
 20 TTGGGAGATG
 1321 GCTCGTCGTT GTATCACAGG AGGGATCGTG GAAGAATACC AATTGCCATA
 TTACAACATG
 1381 GTACCGAGTG ATCCGTCATA CGAAGATATG CGTGAGGTTG TGTGTGTCAA
 ACGTTTGCGG
 25 1441 CCAATTGTGT CTAATCGGTG GAACAGTGAT GAATGTCTAC GAGCAGTTTT
 GAAGCTAATG
 1501 TCAGAATGCT GGGCCCACAA TCCAGCCTCC AGACTCACAG CATTGAGAAT
 TAAGAAGACG
 1561 CTTGCCAAGA TGGTTGAATC CCAAGATGTA AAAATC (SEQ ID NO: 24)

30 A nucleic acid sequence encoding the extracellular human ALK3 polypeptide is as
 follows:

1 CAGAATCTGG ATAGTATGCT TCATGGCACT GGGATGAAAT CAGACTCCGA
 CCAGAAAAAG
 61 TCAGAAAATG GAGTAACCTT AGCACCAGAG GATACCTTGC CTTTTTTAAA
 35 GTGCTATTGC

121 TCAGGGCACT GTCCAGATGA TGCTATTAAT AACACATGCA TAACTAATGG
ACATTGCTTT

181 GCCATCATAG AAGAAGATGA CCAGGGAGAA ACCACATTAG CTTCAGGGTG
TATGAAATAT

5 241 GAAGGATCTG ATTTTCAGTG CAAAGATTCT CAAAAGCCC AGCTACGCCG
GACAATAGAA

301 TGTGTGTCGGA CCAATTTATG TAACCAGTAT TTGCAACCCA CACTGCCCCC
TGTTGTCATA

361 GGTCCGTTTT TTGATGGCAG CATTCGA (SEQ ID NO: 25)

10 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.

15 In certain embodiments, the disclosure relates to single-arm heteromultimer complexes 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.*, single-arm heteromultimer complexes comprising an ALK3 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain
20 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, single-arm heteromultimer complexes of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of an amino acid
25 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, single-arm heteromultimer complexes of the disclosure comprise at least one ALK3 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99%
30 identical to the amino acid sequence of SEQ ID NO: 22, 23, 122, 124, 415, or 416. In some embodiments, single-arm 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, 122, 124, 415, or 416.

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. Numbering of amino acids for all ALK4-related polypeptides described herein is based on the numbering of the human ALK4 precursor protein sequence below (SEQ ID NO: 26), unless specifically designated otherwise.

The protein sequence of canonical human ALK4 precursor (isoform A, NCBI Ref Seq NP_004293) is as follows:

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1  MAESAGASSF FPLVVLLLAG SGGSGPRGVQ ALLCACTSCL QANYTCETDG
ACMVSIFNLD
61  GMEHHVRTCI PKVELVPAGK PFYCLSSIDL RNTHCCYTDY CNRIDLRVPS
20  GHLKEPEHPS
121  MWGPVELVGI IAGPVFLLFL IIIIVFLVIN YHQRVYHNRQ RLDMEDPSCE
MCLSKDKTLQ
181  DLVYDLSTSG SGSGPLPLFVQ RTVARTIVLQ EIIGKGRFGE VWRGRWRGGD
VAVKIFSSRE
25  241  ERSWFREAEI YQTVMLRHEN ILGFIAADNK DNGTWTQLWL VSDYHEHGSL
FDYLNRYTVT
301  IEGMIKLALS AASGLAHLHM EIVGTQ GKPG IAHRDLKSKN ILVKKNGMCA
IADLGLAVRH
361  DAVTDTIDIA PNQRVG TKRY MAPEVLDETI NMKHFDSFKC ADIYALGLVY
30  WEIARRCNSG
421  GVHEEYQLPY YDLVPSDPSI EEMRKVCDQ KLRPNIPNWW QSYEALRVMG
KMMRECWYAN
481  GAARLTALRI KKTLSQLSVQ EDVKI (SEQ ID NO: 26)

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

The processed extracellular human ALK4 polypeptide sequence is as follows:

SGPRGVQALLCACTSCLOANYTCETDGACMVSI FNLDGMEHHVRTCIPKVELVPAGKPFYCL
 5 SSEDLRNTHCCYTDYCNRIDLRVPSGHLKEPEHPSMWGPVE (SEQ ID NO: 27)

A nucleic acid sequence encoding the ALK4 precursor protein is shown below (SEQ ID NO: 28), corresponding to nucleotides 78-1592 of Genbank Reference Sequence NM_004302.4. The signal sequence is underlined and the extracellular domain is indicated in **bold** font.

10 ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGCCGGCAG
CGGCGGGTCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGG
CCAACTACACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATG
GAGCACCATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTA
CTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGA
 15 **TCGACTTGAGGGTGCCCAGTGGTCACTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCC**
GTGGAGCTGGTAGGCATCATCGCCGGCCCGGTGTTCCCTCCTGTTCCCTCATCATCATCATTGT
 TTTCTTGTTCATTAACATATCATCAGCGTGTCTATCACAACCGCCAGAGACTGGACATGGAAG
 ATCCCTCATGTGAGATGTGTCTCTCCAAAGACAAGACGCTCCAGGATCTTGTCTACGATCTC
 TCCACCTCAGGGTCTGGCTCAGGGTTACCCCTCTTTGTCCAGCGCACAGTGGCCCCGAACCAT
 20 CGTTTTACAAGAGATTATTGGCAAGGGTCGGTTTGGGGAAGTATGGCGGGGCCGCTGGAGGG
 GTGGTGATGTGGCTGTGAAAATATTCTCTTCTCGTGAAGAACGGTCTTGTTTCAGGGAAGCA
 GAGATATAACAGACGGTCATGCTGCGCCATGAAACATCCTTGGAATTTATTGCTGCTGACAA
 TAAAGATAATGGCACCTGGACACAGCTGTGGCTTGTTTCTGACTATCATGAGCACGGGTCCC
 TGTTTGATTATCTGAACCGGTACACAGTGACAATTGAGGGGATGATTAAGCTGGCCTTGTCT
 25 GCTGCTAGTGGGCTGGCACACCTGCACATGGAGATCGTGGGCACCCAAGGGAAGCCTGGAAT
 TGCTCATCGAGACTTAAAGTCAAAGAACATTCTGGTGAAGAAAAATGGCATGTGTGCCATAG
 CAGACCTGGGCCTGGCTGTCCGTCATGATGCAGTCACTGACACCATTGACATTGCCCCGAAT
 CAGAGGGTGGGGACCAAACGATACATGGCCCCTGAAGTACTTGATGAAACCATTAATATGAA
 ACACTTTGACTCCTTTAAATGTGCTGATATTTATGCCCTCGGGCTTGTATATTGGGAGATTG
 30 CTCGAAGATGCAATTCTGGAGGAGTCCATGAAGAATATCAGCTGCCATATTACGACTTAGTG
 CCCTCTGACCCTTCCATTGAGGAAATGCGAAAGGTTGTATGTGATCAGAAGCTGCGTCCCAA
 CATCCCCAACTGGTGGCAGAGTTATGAGGCACTGCGGGTGATGGGGAAGATGATGCGAGAGT

GTTGGTATGCCAACGGCGCAGCCCGCCTGACGGCCCTGCGCATCAAGAAGACCCTCTCCCAG
CTCAGCGTGCAGGAAGACGTGAAGATC (SEQ ID NO: 28)

A nucleic acid sequence encoding the extracellular ALK4 polypeptide is as follows:

TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTA
5 CACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACC
ATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTACTGCCTG
AGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGATCGACTT
GAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCCGGTGGAG
(SEQ ID NO: 29)

10 An alternative isoform of human ALK4 precursor, isoform B (NCBI Ref Seq
NP_064732.3), is as follows:

1 **MVSIFNLDGM EHHVRTCIPK VELVPAGKPF YCLSSDLRN THCCYTDYCN**
RIDLRVPSGH

61 **LKEPEHPSMW GPVELVGIIA** GPVFLFLII IIVFLVINYH QRVYHNRQRL
15 DMEDPSCMC
121 LSKDKTLQDL VYDLSTSGSG SGLPLFVQRT VARTIVLQEI IGKGRFGEVW
RGRWRGGDVA
181 VKIFFSSREER SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS
DYHEHGSLEFD
20 241 YLNRYTVTIE GMIKLALSAA SGLAHLHMEI VGTQGKPGIA HRDLKSKNIL
VKKNGMCAIA
301 DLGLAVRHDA VTDITIDIAPN QRVGTRKRYMA PEVLDETINM KHFDSEFKCAD
IYALGLVYWE
361 IARRCNSGGV HEEYQLPYD LVPSDPSIEE MRKVVCQKL RPNIPNWWQS
25 YEALRVMGKM
421 MRECWYANGA ARLTALRIKK TLSQLSVQED VKI (SEQ ID NO: 83)

The extracellular domain is indicated in **bold** font.

The extracellular ALK4 polypeptide sequence (isoform B) is as follows:

MVSIFNLDGMEHHVRTCIPK**VELVPAGKPFYCLSSDLRNTHCCYTDYCN**RIDLRVPSGHLK
30 EPEHPSMWGPVE (SEQ ID NO: 84)

A nucleic acid sequence encoding isoform B of the ALK4 precursor protein is shown
below (SEQ ID NO: 85), corresponding to nucleotides 186-1547 of Genbank Reference
Sequence NM_020327.3. The extracellular domain is indicated in **bold** font.

ATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACCATGTGCGCACCTGCATCCCCAAAGT
 GGAGCTGGTCCCTGCCGGAAGCCCTTCTACTGCCTGAGCTCGGAGGACCTGCGCAACACCC
 ACTGCTGCTACACTGACTACTGCAACAGGATCGACTTGAGGGTGTCCAGTGGTCACCTCAAG
 GAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAGCTGGTAGGCATCATCGCCGGCCCGGT
 5 GTTCCTCCTGTTCTCATCATCATCATTGTTTTCTTGTTCATTAACATCATCAGCGTGTCT
 ATCACAACCGCCAGAGACTGGACATGGAAGATCCCTCATGTGAGATGTGTCTCTCCAAAGAC
 AAGACGCTCCAGGATCTTGTCTACGATCTCTCCACCTCAGGGTCTGGCTCAGGGTTACCCCT
 CTTTGTCCAGCGCACAGTGGCCCGAACCATCGTTTTACAAGAGATTATTGGCAAGGGTCGGT
 TTGGGGAAGTATGGCGGGGCCGCTGGAGGGGTGGTGATGTGGCTGTGAAAATATTCTCTTCT
 10 CGTGAAGAACGGTCTTGGTTCAGGGAAGCAGAGATATACCAGACGGTCATGCTGCGCCATGA
 AAACATCCTTGGATTTATTGCTGCTGACAATAAAGATAATGGCACCTGGACACAGCTGTGGC
 TTGTTTCTGACTATCATGAGCACGGGTCCCTGTTTGATTATCTGAACCGGTACACAGTGACA
 ATTGAGGGGATGATTAAGCTGGCCTTGTCTGCTGCTAGTGGGCTGGCACACCTGCACATGGA
 GATCGTGGGCACCCAAGGGAAGCCTGGAATTGCTCATCGAGACTTAAAGTCAAAGAACATTC
 15 TGGTGAAGAAAAATGGCATGTGTGCCATAGCAGACCTGGGCCTGGCTGTCCGTCATGATGCA
 GTCAGTACACCATTTGACATTGCCCCGAATCAGAGGGTGGGGACCAAACGATACATGGCCCC
 TGAAGTACTTGATGAAACCATTAATATGAAACACTTTGACTCCTTTAAATGTGCTGATATTT
 ATGCCCTCGGGCTTGTATATTGGGAGATTGCTCGAAGATGCAATTCTGGAGGAGTCCATGAA
 GAATATCAGCTGCCATATTACGACTTAGTGCCCTCTGACCCTTCCATTGAGGAAATGCGAAA
 20 GGTTGTATGTGATCAGAAGCTGCGTCCCAACATCCCCAACTGGTGGCAGAGTTATGAGGCAC
 TGCGGGTGATGGGGAAGATGATGCGAGAGTGTTGGTATGCCAACGGCGCAGCCCGCCTGACG
 GCCCTGCGCATCAAGAAGACCCTCTCCCAGCTCAGCGTGCGAGGAAGACGTGAAGATC (SEQ
 ID NO: 85)

A nucleic acid sequence encoding the extracellular domain of ALK4 polypeptide
 25 (isoform B) is as follows:

ATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACCATGTGCGCACCTGCATCCCCAAAGT
 GGAGCTGGTCCCTGCCGGAAGCCCTTCTACTGCCTGAGCTCGGAGGACCTGCGCAACACCC
 ACTGCTGCTACACTGACTACTGCAACAGGATCGACTTGAGGGTGTCCAGTGGTCACCTCAAG
 GAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAG (SEQ ID NO: 86)

30 In certain embodiments, the disclosure relates to single-arm heteromultimer
 complexes 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.*, single-arm heteromultimer complexes

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, single-arm heteromultimer complexes of the disclosure comprise at least one ALK4 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: 26, 27, 83, 84, 125, 127, 417, or 418. In some embodiments, single-arm heteromultimer complexes 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%, or 99% identical to the amino acid sequence of SEQ ID NO: 26, 27, 83, 84, 125, 127, 417, or 418.

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. Numbering of amino acids for all ALK5-related polypeptides described herein is based on the numbering of the human ALK5 precursor protein sequence below (SEQ ID NO: 30), unless specifically designated otherwise.

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

```

1  MEAAVAAPRP  RLLLLVLAAA  AAAAAALLPG  ATALQCFCHL  CTKDNFTCVT
DGLCFVSVTE
61  TTDKVIHNSM  CIAEIDLIPR  DRPFVCAPSS  KTGSVTTTYC  CNQDHCNKIE
LPTTVKSSPG
121  LGPVELAAVI  AGPVCFVCIS  LMLMVYICHN  RTVIHHRVPN  EEDPSLDRPF
ISEGTTLKDL

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181 IYDMTTSGSG SGLPLLVRT IARTIVLQES IGKGRFGEVW RGKWRGEEVA
VKIFSSREER
241 SWFREAEIYQ TVMLRHENIL GFIAADNKN GTWTQLWLVS DYHEHGSLEFD
YLNRYTVTVE
5 301 GMIKLALSTA SGLAHLHMEI VGTQGKPAIA HRDLKSKNIL VKKNGTCCIA
DLGLAVRHDS
361 ATDTIDIAPN HRVGTKRYMA PEVLDD SINM KHFESFKRAD IYAMGLVFWE
IARRCSIGGI
421 HEDYQLPYD LVPSDPSVEE MRKVVCEQKL RPNIPNRWQS CEALRVMAKI
10 MRECWYANGA
481 ARLTALRIKK TLSQLSQEG IKM (SEQ ID NO: 30)

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

The processed extracellular ALK5 polypeptide sequence is as follows:

15 AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPDRPFVC
APSSKTGSVTTTTYCCNQDHCNKIELPTTVKSSPGLGPVEL (SEQ ID NO: 31)

A nucleic acid sequence encoding the ALK5 precursor protein is shown below (SEQ ID NO: 32), corresponding to nucleotides 77-1585 of Genbank Reference Sequence NM_004612.2. The signal sequence is underlined and the extracellular domain is indicated in **bold font**.

ATGGAGGCGGCGGTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGC
GGCGGCGGCGGCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTA
CAAAAGACAATTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACA
GACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCC
25 **GTTTGTATGTGCACCCTCTTCAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGG**
ACCATTGCAATAAAATAGAACTTCCAAC TACTGTAAAGTCATCACCTGGCCTTGGTCCTGTG
GAACTGGCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACTCATGTTGATGGT
CTATATCTGCCACAACCGCACTGTCATTCAACATCGAGTGCCAAATGAAGAGGACCCTTCAT
TAGATCGCCCTTTTATTTTCAAGGGTACTACGTTGAAAGACTTAATTTATGATATGACAACG
30 TCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAACAATTGCGAGAACTATTGTGTT
ACAAGAAAGCATTTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGGGGAGAAG
AAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTTCGTGGTTCCGTGAGGCAGAGATT
TATCAAAC TGTAAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGCAGACAATAAAGA

CAATGGTACTTGGACTCAGCTCTGGTTGGTGTGAGATTATCATGAGCATGGATCCCTTTTGG
 ATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAACTTGCTCTGTCCACGGCG
 AGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGAAAGCCAGCCATTGCTCA
 TAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACCTTGCTGTATTGCAGACT
 5 TAGGACTGGCAGTAAGACATGATTGAGCCACAGATACCATTGATATTGCTCCAAACACAGA
 GTGGGAACAAAAAGGTACATGGCCCCCTGAAGTTCTCGATGATTCCATAAATATGAAACATTT
 TGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCTGGGAAATTGCTCGAC
 GATGTTCCATTGGTGGAAATTCATGAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCT
 GACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAGGCCAAATATCCC
 10 AAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGAGAGAATGTTGGT
 ATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATTAAGAAAACATTATCGCAACTCAGT
 CAACAGGAAGGCATCAAAATG (SEQ ID NO: 32)

A nucleic acid sequence encoding the extracellular human ALK5 polypeptide is as follows:

15 GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
 TTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACAGACAAAGTTA
 TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
 GCACCCTCTTCAAAAACCTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
 TAAAATAGAACTTCCAACACTGTAAAGTCATCACCTGGCCTTGGTCCTGTGGAAGT
 20 (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 AAAAAALLPG **ATALQCFCHL CTKDNFTCVT**
 25 **DGLCFVSVTE**
 61 **TTDKVIHNSM CIAEIDLIPR DRPFVCPASS KTGSVTTTYC CNQDHCNKIE**
LPTTGPFVSVK
 121 **SSPGLGPVEL** AAVIAGPVCF VCISLMLMVY ICHNRTVIHH RVPNEEDPSL
 DRPFISEGTT
 30 181 LKDLIYDMMT SGSGSGLPLL VQRTIARTIV LQESIGKGRF GEVWRGKWRG
 EEVAVKIFSS
 241 REERSWFREA EIIQTVMLRH ENILGFIAAD NKDNGTWTQL WLVS DYHEHG
 SLFDYLNRYT
 301 VTVEGMIKLA LSTASGLAHL HMEIVGTQGK PAIAHRDLKS KNILVKKNGT
 35 CCIADLGLAV

361 RHDSATDTID IAPNHRVGTK RYMAPEVLDD SINMKHFESF KRADIYAMGL
 VFWEIARRCS
 421 IGGIHEDYQL PYYDLVPSDP SVEEMRKVVC EQKLRPNIPN RWQSCEALRV
 MAKIMRECWY

5 481 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**.

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

AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPDRPFVC
 10 APSSKTGSVTTTTYCCNQDHCNKIELPTTGPFVSVKSSPGLGPVEL (SEQ ID NO: 88)

A nucleic acid sequence encoding human ALK5 precursor protein (isoform 2) is shown below (SEQ ID NO: 89), corresponding to nucleotides 77-1597 of Genbank Reference Sequence XM_005252150.1. The signal sequence is underlined and the extracellular domain is indicated in **bold font**.

15 ATGGAGGCGGCGGTTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGC
GGCGGCGGCGGCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTA
CAAAAGACAATTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACA
GACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCC
GTTTGTATGTGCACCTCTTCAAAAACCTGGGTCTGTGACTACAACATATTGCTGCAATCAGG
 20 **ACCATTGCAATAAAATAGAACTTCCAACCTACTGGCCCTTTTTTCAGTAAAGTCATCACCTGGC**
CTTGGTCTGTGGAACCTGGCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACT
CATGTTGATGGTCTATATCTGCCACAACCGCACTGTCATTCACCATCGAGTGCCAAATGAAG
AGGACCCCTTCATTAGATCGCCCTTTTATTTTCAGAGGGTACTACGTTGAAAGACTTAATTTAT
GATATGACAACGTCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTTCAGAGAACAATTGCGAG
 25 **AACTATTGTGTTACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGT**
GGCGGGGAGAAGAAGTTGCTGTAAAGATATTCTCCTCTAGAGAAGAACGTTTCGTGGTTCCGT
GAGGCAGAGATTTATCAAACCTGTAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGC
AGACAATAAAGACAATGGTACTTGGACTCAGCTCTGGTTGGTGTGAGATTATCATGAGCATG
GATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAACTTGCT
 30 **CTGTCCACGGCGAGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGAAAGCC**
AGCCATTGCTCATAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACCTTGCT
GTATTGCAGACTTAGGACTGGCAGTAAGACATGATTCAGCCACAGATACCATTGATATTGCT
CCAAACCACAGAGTGGGAACAAAAGGTACATGGCCCCTGAAGTTCTCGATGATTCCATAAA

TATGAAACATTTTGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCTGGG
 AAATTGCTCGACGATGTTCCATTGGTGGAAATTCATGAAGATTACCAACTGCCTTATTATGAT
 CTTGTACCTTCTGACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAG
 GCCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGA
 5 GAGAATGTTGGTATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATTAAGAAAACATTA
 TCGCAACTCAGTCAACAGGAAGGCATCAAAATG (SEQ ID NO: 89)

A nucleic acid sequence encoding the processed extracellular ALK5 polypeptide is as follows:

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
 10 TTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACAGACAAAGTTA
 TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCCTCGAGATAGGCCGTTTGTATGT
 GCACCCTCTTCAAAAACCTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
 TAAAATAGAACTTCCAACACTACTGGCCCTTTTTCAGTAAAGTCATCACCTGGCCTTGGTCCTG
 TGGAACCTG (SEQ ID NO: 90)

15 In certain embodiments, the disclosure relates to single-arm heteromultimer complexes 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.*, single-arm heteromultimer complexes comprising an ALK5 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain
 20 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, single-arm heteromultimer complexes of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99%
 25 identical to the amino acid sequence of SEQ ID NO: 30, 31, 87, 88, 128, 130, 419, or 420. In some embodiments, single-arm 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%, or 99% identical to the amino acid sequence of SEQ ID NO: 30, 31, 87, 88, 128, 130, 419, or 420.

30 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. Numbering of amino acids for all ALK6-related polypeptides described herein is based on the numbering of the human ALK6 precursor protein sequence below (SEQ ID NO: 34), unless specifically designated otherwise.

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

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

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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:

KKEDGESTAPTPRPKVLRCCKHHHCPEDSVNNICSTDGYCFTMIEEDDSGLPVVTSGLGLE
 GSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFVDGPIHHR (SEQ ID
 NO: 35)

A nucleic acid sequence encoding the ALK6 precursor protein is shown below (SEQ
 ID NO: 36), corresponding to nucleotides 275-1780 of Genbank Reference Sequence
 NM_001203.2. The signal sequence is underlined and the extracellular domain is indicated
 in **bold** font.

ATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACC**AAGAAAGAGGATGGTGAGAGTAC**
AGCCCCCAGCCCCCGTCCAAAGGTCTTGCGTTGTAAATGCCACCACCATTGTCCAGAAGACT
CAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCACGATGATAGAAGAGGATGACTCT
GGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAAGGCTCAGATTTTCAGTGTGCGGA
CACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAAGGAACGAATGTAATA
AAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTGATGGACCTATACAC
CACAGGGCTTTACTTATATCTGTGACTGTCTGTAGTTTGCTCTTGGTCCTTATCATATTATT
TTGTTACTTCCGGTATAAAAGACAAGAAACCAGACCTCGATACAGCATTGGGTTAGAACAGG
ATGAAACTTACATTCCCTCCTGGAGAATCCCTGAGAGACTTAATTGAGCAGTCTCAGAGCTCA
GGAAGTGGATCAGGCCTCCCTCTGCTGGTCCAAAGGACTATAGCTAAGCAGATTCAGATGGT
GAAACAGATTGGAAAAGGTGCTATGGGGAAGTTTGATGGGAAAGTGGCGTGGCGAAAAGG
TAGCTGTGAAAGTGTTCTTCACCACAGAGGAAGCCAGCTGGTTCAGAGAGACAGAAATATAT
CAGACAGTGTTGATGAGGCATGAAAACATTTTGGGTTTCATTGCTGCAGATATCAAAGGGAC
AGGGTCCTGGACCCAGTTGTACCTAATCACAGACTATCATGAAAATGGTTCCCTTTATGATT
ATCTGAAGTCCACCACCCTAGACGCTAAATCAATGCTGAAGTTAGCCTACTCTTCTGTCAGT
GGCTTATGTCATTTACACACAGAAATCTTTAGTACTCAAGGCAAACCAGCAATTGCCCATCG
AGATCTGAAAAGTAAAAACATTCTGGTGAAGAAAAATGGAACCTGCTGTATTGCTGACCTGG
GCCTGGCTGTAAATTTATTAGTGATACAAATGAAGTTGACATACCACCTAACACTCGAGTT
GGCACCAAACGCTATATGCCTCCAGAAGTGTTGGACGAGAGCTTGAACAGAAATCACTTCCA
GTCTTACATCATGGCTGACATGTATAGTTTTGGCCTCATCCTTTGGGAGGTTGCTAGGAGAT
GTGTATCAGGAGGTATAGTGGAAGAATACCAGCTTCCTTATCATGACCTAGTGCCAGTGAC
CCCTCTTATGAGGACATGAGGGAGATTGTGTGCATCAAGAAGTTACGCCCCCTCATTCCCAA
CCGGTGGAGCAGTGATGAGTGTCTAAGGCAGATGGGAAAACATCATGACAGAATGCTGGGCTC
ACAATCCTGCATCAAGGCTGACAGCCCTGCGGGTTAAGAAAACACTTGCCAAAATGTCAGAG
TCCCAGGACATTAAACTC (SEQ ID NO: 36)

A nucleic acid sequence encoding processed extracellular ALK6 polypeptide is as follows:

AAGAAAGAGGATGGTGAGAGTACAGCCCCACCCCCGTCCAAAGGTCTTGCGTTGTAAATG
 CCACCACCATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCA
 5 CGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAA
 GGCTCAGATTTTTCAGTGTCGGGACACTCCCATTCTCATCAAAGAAGATCAATTGAATGCTG
 CACAGAAAGGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAG
 ATTTTGTGATGGACCTATACACCACAGG (SEQ ID NO: 37)

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

1 MGWLEELNWQ LHIFLLILLS MHTRANFLDN **MLLRSAGKLN VGTKKEDGES**
TAPTPRPKVL
 61 **RCKCHHHCPEDSVNNICSTD GYCFTMIEED DSGLPVVTSG CLGLEGSDFQ**
CRDTPIPHQR
 15 121 **RSIECCTERN ECNKDLHPTL PPLKNRDFVD GPIHHRALLI** SVTVCSLLLV
 LIILFCYFRY
 181 KRQETRPRYS IGLEQDETYI PPGESLRDLI EQSQSSGSGS GLPLLVQRTI
 AKQIQMVKQI
 241 GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT VLMRHENILG
 20 FIAADIKGTG
 301 SWTQLYLITD YHENGSLYDY LKSTTLDAKS MLKLAYSSVS GLCHLHTEIF
 STQGKPAIAH
 361 RDLKSKNILV KNGTCCIA D LGLAVKFISD TNEVDIPPNT RVGTKRYMPP
 EVLDES LN RN
 25 421 HFQSYIMADM YSFGILILWEV ARRCVSGGIV EEYQLPYHDL VPSDPSYEDM
 REIVCIKKLR
 481 PSFPNRWSSD ECLRQMGKLM TECWAHNPAS RLALRVKKT LAKMSESQDI KL (SEQ
 ID NO: 91)

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

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

NFLDNMLLRSAGKLVGTTKEDGESTAPTPRPKVL RCKCHHHCPEDSVNNICSTDGYCFTMI
 EEDDSGLPVVTSGCLGLEGSDFQCRDTPIPHQRRSIECCTERN ECNKDLHPTLPPLKNRDFV
 DGPIHHR (SEQ ID NO: 92)

A nucleic acid sequence encoding human ALK6 precursor protein (isoform 2) is shown below, corresponding to nucleotides 22-1617 of Genbank Reference Sequence NM_001256793.1. The signal sequence is underlined and the extracellular domain is indicated in **bold** font.

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5  ATGGGTTGGCTGGAAGAACTAAACTGGCAGCTTCACATTTTCTTGCTCATTCTTCTCTCTAT
   GCACACAAGGGCAAACTTCCTTGATAACATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGG
   GCACCAAGAAAGAGGATGGTGAGAGTACAGCCCCACCCCCCGTCCAAAGGTCTTGCGTTGT
   AAATGCCACCACCATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTG
   TTTCACGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGAC
10 TAGAAGGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCCTCATCAAAGAAGATCAATTGAA
   TGCTGCACAGAAAGGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAA
   CAGAGATTTTGTGATGGACCTATACACCACAGGGCTTTACTTATATCTGTGACTGTCTGTA
   GTTTGCTCTTGGTCCTTATCATATTATTTTGTACTTCCGGTATAAAAGACAAGAAACCAGA
   CCTCGATACAGCATTGGGTTAGAACAGGATGAACTTACATTCCCTCCTGGAGAATCCCTGAG
15 AGACTTAATTGAGCAGTCTCAGAGCTCAGGAAGTGGATCAGGCCTCCCTCTGCTGGTCCAAA
   GGACTATAGCTAAGCAGATTCAGATGGTGAAACAGATTGGAAAAGGTGCGTATGGGGAAGTT
   TGGATGGGAAAGTGGCGTGGCGAAAAGGTAGCTGTGAAAGTGTTCTTCACCACAGAGGAAGC
   CAGCTGGTTCAGAGAGACAGAAATATATCAGACAGTGTTGATGAGGCATGAAAACATTTTGG
   GTTTCATTGCTGCAGATATCAAAGGGACAGGGTCTGGACCCAGTTGTACCTAATCACAGAC
20 TATCATGAAAATGGTTCCCTTTATGATTATCTGAAGTCCACCACCCTAGACGCTAAATCAAT
   GCTGAAGTTAGCCTACTCTTCTGTGTCAGTGGCTTATGTCATTTACACACAGAAATCTTTAGTA
   CTCAAGGCAAACCAGCAATTGCCCATCGAGATCTGAAAAGTAAAAACATTCTGGTGAAGAAA
   AATGGAACCTTGCTGTATTGCTGACCTGGGCCTGGCTGTTAAATTTATTAGTGATACAAATGA
   AGTTGACATACCACCTAACACTCGAGTTGGCACCAAACGCTATATGCCTCCAGAAGTGTTGG
25 ACGAGAGCTTGAACAGAAATCACTTCCAGTCTTACATCATGGCTGACATGTATAGTTTTGGC
   CTCATCCTTTGGGAGGTTGCTAGGAGATGTGTATCAGGAGGTATAGTGGAAGAATACCAGCT
   TCCTTATCATGACCTAGTGCCCAGTGACCCCTCTTATGAGGACATGAGGGAGATTGTGTGCA
   TCAAGAAGTTACGCCCCTCATTCCTCAAACCGGTGGAGCAGTGATGAGTGTCTAAGGCAGATG
   GGAAAACATCATGACAGAATGCTGGGCTCACAAATCCTGCATCAAGGCTGACAGCCCTGCGGGT
30 TAAGAAAACACTTGCCAAAATGTCAGAGTCCCAGGACATTAACTC (SEQ ID NO: 93)

```

A nucleic acid sequence encoding the processed extracellular ALK6 polypeptide is as follows:

AACTTCCTTGATAACATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACCAAGAAAGA
 GGATGGTGAGAGTACAGCCCCCACCCTCCAAAGGTCTTGCGTTGTAAATGCCACCACC
 ATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCACGATGATA
 GAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAAGGCTCAGA
 5 TTTTCAGTGTCGGGACACTCCCATTCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAA
 GGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTT
 GATGGACCTATACACCACAGG (SEQ ID NO: 94)

In certain embodiments, the disclosure relates to single-arm heteromultimer complexes that comprise at least one ALK6 polypeptide, which includes fragments,
 10 functional variants, and modified forms thereof. Preferably, ALK6 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, single-arm heteromultimer complexes 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
 15 Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, single-arm heteromultimer complexes of the disclosure comprise at least one ALK6 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: 34, 35, 91, 92, 131, 133, 421, or 422. In some embodiments, single-arm heteromultimer complexes of the disclosure consist or consist
 20 essentially of at least one ALK6 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: 34, 35, 91, 92, 131, 133, 421, or 422.

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-
 25 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
 30 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.

Numbering of amino acids for all ALK7-related polypeptides described herein is based on the numbering of the human ALK7 precursor protein sequence below (SEQ ID NO: 38), unless specifically designated otherwise.

Several naturally occurring isoforms of human ALK7 have been described. The sequence of canonical 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
10 PMELAIITV
121 PVCLLSIAAM LTVWACQGRQ CSYRKKRPN VEEPLSECNL VNAGKTLKDL
IYDVTASGSG
181 SGLPLLQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER
SWFREAEIYQ
15 241 TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA
GMIKLALSIA
301 SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS
ILNTIDIPQN
361 PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI
20 VEEYQLPYD
421 MVPSDPSIEE MRKVVCQKF 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.

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

```

ELSPGLKCVCLLCDSSNFTCQTEGACWASVMLTNGKEQVIKSCVSLPELNAQVFCHSSNNVT
KTECCFTDFCNNITLHLPTASPNAPKLGPM (SEQ ID NO: 39)

```

A nucleic acid sequence encoding human ALK7 isoform 1 precursor protein is shown below (SEQ ID NO: 40), corresponding to nucleotides 244-1722 of Genbank Reference Sequence NM_145259.2. The signal sequence is underlined and the extracellular domain is indicated in **bold** font.

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGA
 GCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACCTTTACCTGCCAAA
 CAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCC
 TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAA
 5 AACCGAATGCTGCTTCACAGATTTTTGCAACAACATAAACACTGCACCTTCCAACAGCATCAC
 CAAATGCCCCAAAACCTTGGACCCATGGAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTC
 CTGTCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTGCTCCTACAGGAA
 GAAAAAGAGACCAAATGTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTGGAAAAA
 CTCTGAAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTG
 10 GTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGG
 TGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAG
 ATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAAC
 ATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAACCTTGGACTCAACTTTGGCTGGT
 ATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGG
 15 CTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATT
 GTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGT
 GAAAAAGTGTGAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATAC
 TGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAA
 ATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTC
 20 TGTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTTCAGTCGGAGGAATTGTTGAGGAGT
 ACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTT
 GTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACAGTGGCAGAGTTGTGAAGCACTCCG
 AGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTC
 TTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC (SEQ ID
 25 NO: 40)

A nucleic acid sequence encoding the processed extracellular ALK7 polypeptide (isoform 1) is as follows:

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACCTTTACCTGCCA
 AACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAAT
 30 CCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
 AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAAACACTGCACCTTCCAACAGCATC
 ACCAAATGCCCCAAAACCTTGGACCCATGGAG (SEQ ID NO: 41)

The 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
5  TASPNAPKLG
    61  PMELAIITV PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL
VNAGKTLKDL
    121  IYDVTASGSG SGLPLLVRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA
VKIFSSRDER
10   181  SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD
YLNRNIVTVA
    241  GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA
DLGLAVKHDS
    301  ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE
15  IARRCSVGGI
    361  VEEYQLPYD MVPSDPSIEE MRKVCDQKF RPSIPNQWQS CEALRVMGRI
MRECWYANGA
    421  ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 301)

```

The 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 (SEQ ID NO: 303), corresponding to nucleotides 279-1607 of NCBI Reference Sequence NM_001111031.1. The extracellular domain is indicated in **bold** font.

```

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGT
CTTCTGTCTAGTTCCAACAATGTTACCAAACCGAATGCTGCTTCACAGATTTTTCACAACACATAA
CACCTGCACCTTCCAACAGCATCACCAAATGCCCCAAACTTGGACCCATGGAGCTGGCCATCATTATT
ACTGTGCCTGTTTGCCTCCTGTCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTG
30  CTCTTACAGGAAGAAAAAGAGACCAAATGTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTG
GAAAACTCTGAAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTG
GTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGT
GTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTT
GGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCT
35  GCTGACAACAAAGATAATGGAACCTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTC

```

CTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTG
 CTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGA
 GACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGC
 TGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGT
 5 ATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGAC
 ATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTTCAGTCGGAGGAATTGTTGAGGA
 GTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTT
 GTGACCAGAAGTTTCGACCAAGTATCCCAAACAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGG
 AGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGAC
 10 TATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 303)

A nucleic acid sequence encoding the extracellular ALK7 polypeptide (isoform 2) is as follows (SEQ ID NO: 304):

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGT
 CTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAA
 15 CACTGCACCTTCCAACAGCATCACCAAATGCCCCAAACTTGGACCCATGGAG (SEQ ID NO:
 304)

The 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.

20 1 MTRALCSALR QALLLLAAAA ELSPGLKVCV LLCDSNFTC QTEGACWASV
 MLTNGKEQVI
 61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TGLPLLQRT
 IARTIVLQEI
 121 VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ TVMLRHENIL
 25 GFIAADNKDN
 181 GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA SGLAHLHMEI
 VGTQGKPAIA
 241 HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA
 PEMLDDTMNV
 30 301 NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYD MVPSDPSIEE
 MRKVCDQKF
 361 RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK TISQLCVKED CKA
 (SEQ ID NO: 305)

The amino acid sequence of the processed ALK7 polypeptide (isoform 3) is as
 35 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 explained below.

```

1  ELSPGLKCVC  LLCSSNFTC  QTEGACWASV  MLTNGKEQVI  KSCVSLPELN
A QV FCHSSNN
5  61  VTKTECCFTD  FCNNITLHLP  TGLPLLVQRT  IARTIVLQEI  VGKGRFGEVW
HGRWCGEDVA
121 VKIFSSRDER  SWFREAEIYQ  TVMLRHENIL  GFIAADNKDN  GTWTQLWLVS
EYHEQGSLYD
181 YLNRNIVTVA  GMIKLALSIA  SGLAHLHMEI  VGTQGKPAIA  HRDIKSKNIL
10 VKKCETCAIA
241 DLGLAVKHDS  ILNTIDIPQN  PKVGTKRYMA  PEMLDDTMNV  NIFESFKRAD
IYSVGLVYWE
301 IARRCSVGGI  VEEYQLPYD  MVPSDPSIEE  MRKVVCQKF  RPSIPNQWQS
CEALRVMGRI
15 361 MRECWYANGA  ARLTALRIKK  TISQLCVKED  CKA  (SEQ ID NO: 306)

```

A nucleic acid sequence encoding the unprocessed ALK7 polypeptide precursor protein (isoform 3) is shown below (SEQ ID NO: 307), corresponding to nucleotides 244-1482 of NCBI Reference Sequence NM_001111032.1. The signal sequence is indicated by solid underline.

```

20  ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGAGCTCTC
GCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACCTTTACCTGCCAAACAGAAGGAGCAT
GTTGGGCATCAGTCATGCTAACCAATGAAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAA
CTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAACCGAATGCTGCTTCACAGATTT
TTGCAACAACATAACACTGCACCTTCCAACAGGTCTACCTCTGTTGGTTCAAAGGACAATTGCAAGGA
25  CGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGG
GAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTA
CCAGACGGTCATGCTGCGACATGAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAA
CTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGA
AATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCA
30  TATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCT
TAGTGAAAAAGTGTGAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTG
AACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGA
TGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTT
ACTGGGAAATAGCCCGGAGGTGTTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGAC
35  ATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAG
TATCCCAAACCAAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGT

```

ATGCCAACGGAGCGGCCCCGCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAA
GAAGACTGCAAAGCC (SEQ ID NO: 307)

A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 3) is as follows (SEQ ID NO: 308):

5 GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACCTTTACCTGCCAAACAGA
AGGAGCATGTTGGGCATCAGTCATGCTAACCAATGAAAAAGAGCAGGTGATCAAATCCTGTGTCTCCC
TTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTC
ACAGATTTTTTGCAACAACATAACACTGCACCTTCCAACAGGTCTACCTCTGTTGGTTCAAAGGACAAT
TGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGAT
10 GGTGTGGGGAAGATGTGGCTGTGAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCA
GAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGA
TAATGGAACCTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATT
TGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCA
CACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAA
15 GAATATCTTAGTGAAAAAGTGTGAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATT
CAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAA
ATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGG
TCTGGTTTACTGGGAAATAGCCCGGAGGTGTTGAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTT
ATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTT
20 CGACCAAGTATCCCAAACAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGA
GTGTTGGTATGCCAACGGAGCGGCCCCGCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTT
GTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 308)

The 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.

25 1 MTRALCSALR QALLLLAAAA ELSPGLKVCV LCDSSNFTC QTEGACWASV
MLTNGKEQVI
61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TDNGTWTQLW
LVSEYHEQGS
30 121 LYDYLNRNIV TVAGMIKLAL SIASGLAHLH MEIVGTQGKP AIAHRDIKSK
NILVKKCETC
181 AIADLGLAVK HDSILNTIDI PQNPKVGTKR YMAPEMLDDT MNVNIFESFK
RADIYSVGLV
241 YWEIARRCSV GGIVEEYQLP YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ
35 WQSCEALRVM

301 GRIMRECWYA NGAARLTALR IKKTISQLCV KEDCKA (SEQ ID NO: 309)

The amino acid sequence of the 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 5 68:1719-1726). N-terminal variants of SEQ ID NO: 310 are predicted as explained below.

1 ELSPGLKCVC LLCSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
AQVFCHSSNN

61 VTKTECCFTD FCNNITLHLP TDNGTWTQLW LVSEYHEQGS LYDYLNRNIV
TVAGMIKLAL

10 121 SIASGLAHLH MEIVGTQGKP AIAHRDIKSK NILVKKCETC AIADLGLAVK
HDSILNTIDI

181 PQNPKVGTRK YMAPEMLDDT MNVNIFESFK RADIYSVGLV YWEIARRCSV
GGIVEEYQLP

240 YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM GRIMRECWYA
15 NGAARLTALR

301 IKKTISQLCV KEDCKA (SEQ ID NO: 310)

A nucleic acid sequence encoding the unprocessed ALK7 polypeptide precursor protein (isoform 4) is shown below (SEQ ID NO: 311), corresponding to nucleotides 244-1244 of NCBI Reference Sequence NM_001111033.1. The signal sequence is indicated by 20 solid underline.

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGCCGCCGAGCTCTC
GCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACCTTTACCTGCCAAACAGAAGGAGCAT
GTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAA
CTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAACCGAATGCTGCTTCACAGATTT
25 TTGCAACAACATAACACTGCACCTTCCAACAGATAATGGAACCTGGACTCAACTTTGGCTGGTATCTG
AATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATC
AAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAA
ACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAACTTGTGCCA
TAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCT
30 AAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGA
GTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTTCAG
TCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAG
GAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACAGTGCCAAAGTTGTGA
AGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTG

CTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCCTAA (SEQ ID NO: 311)

A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 4) is as follows (SEQ ID NO: 312):

5 GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACCTTACCTGCCAAACAGA
AGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCC
TTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTC
ACAGATTTTTTGCAACAACATAACACTGCACCTTCCAACAGATAATGGAACCTTGGACTCAACTTTGGCT
GGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTG
10 GAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACA
CAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAAC
TTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTC
AGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAAT
ATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAG
15 GTGTTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCT
CGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCGAGTGGCAA
AGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCCG
CCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCCTAA
(SEQ ID NO: 312)

20 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).

25 1 LKCVCLLCDS SNFTCQTEGA CWASVMLTNG KEQVIKSCVS LPELNAQVFC
HSSNNVTKTE
61 CCFTDFCNNI TLHLPTASPN APKLGPM (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
30 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 single-arm heteromultimer complexes 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.*, single-arm heteromultimer complexes 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, single-arm heteromultimer complexes of the disclosure comprise at least one ALK7 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: 38, 39, 134, 136, 301, 302, 305, 306, 309, 310, 313, 423, or 424. In some embodiments, single-arm heteromultimer complexes 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%, or 99% identical to the amino acid sequence of SEQ ID NO: 38, 39, 134, 136, 301, 302, 305, 306, 309, 310, 313, 423, or 424.

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) or a TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBR1, 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 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- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, nodal, GDNF, neurturin, artemin, persephin, MIS, and Lefty.

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) or a TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBR2, BMPRII, and MISRII) 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 single-arm 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) or a TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBR2, BMPRII, and MISRII) of the present disclosure, as well as truncation mutants. Pools of combinatorial mutants are especially useful for identifying TGF-beta superfamily type I 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 or type II receptor polypeptide 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- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, 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 receptor single-arm heteromultimer complex of the disclosure also may be tested in a cell-based or *in vivo* assay. For example, the effect of a single-arm heteromultimer complex on the expression of genes 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- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, GDNF, neurturin, artemin, persephin, MIS, and Lefty), and cells may be transfected so as to produce a TGF-beta superfamily type I or type II receptor single-arm complex, and optionally, a TGF-beta superfamily ligand. Likewise, a single-arm 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 TGF-beta superfamily receptor polypeptide or its variants 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 receptor single-arm heteromultimer complex. Such variants, when expressed from recombinant DNA constructs, can be used in gene therapy protocols. Likewise, mutagenesis can give rise to variants which have extracellular half-lives dramatically different than the corresponding unmodified TGF-beta superfamily receptor single-arm heteromultimer complex. 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 outside the cell. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter the half-life of the TGF-beta superfamily receptor single-arm heteromultimer complex.

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 or type 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 or type 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.

5 Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, TGF-beta superfamily receptor single-arm heteromultimer complexes 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
10 *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)
15 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
20 (bioactive) forms of TGF-beta superfamily type I or type 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
25 combinatorial mutagenesis of TGF-beta superfamily receptor single-arm heteromultimer complexes 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
30 relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include binding assays and/or cell-signaling assays for 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- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, GDNF, neurturin, artemin, persephin, MIS, and Lefty).

In certain embodiments, TGF-beta superfamily type I and type II receptor single-arm heteromultimer complexes of the disclosure may further comprise post-translational modifications in addition to any that are naturally present in the TGF-beta superfamily type I or type 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 or type II receptor single-arm heteromultimer complex 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 single-arm heteromultimer complex may be tested as described herein for other single-arm 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 or type II receptor polypeptide.

In certain aspects, the polypeptides disclosed herein may form protein complexes comprising at least one TGF-beta superfamily type I or type II receptor polypeptide associated, covalently or non-covalently, with at least one polypeptide comprising a complementary member of an interaction pair. Preferably, polypeptides disclosed herein form single-arm heterodimeric complexes, although higher order heteromultimeric complexes (heteromultimers) are also included such as, but not limited to, heterotrimers, heterotetramers, and further oligomeric structures (see, *e.g.*, Figure 1). In some embodiments, TGF-beta superfamily type I 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 single-arm polypeptide (*e.g.*, a fusion polypeptide comprising a TGF-beta superfamily type

I receptor polypeptide or TGF-beta superfamily type II receptor polypeptide) and a complementary member of an interaction pair 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

5 heteromultimer (see, *e.g.*, Figure 2).

Many methods known in the art can be used to generate TGF-beta superfamily receptor single-arm complexes of the disclosure. For example, non-naturally occurring disulfide bonds may be constructed by replacing on a first polypeptide (*e.g.*, a fusion polypeptide comprising a TGF-beta superfamily type I or type II receptor polypeptide) a

10 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.*, a complementary member of an interaction pair) 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

15 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,

20 *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

25 second polypeptide, wherein the first polypeptide comprises the amino acid sequence of a TGF-beta superfamily type I or 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 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

30 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 receptor 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: 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. 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 interaction-pair complex (see, e.g., Figure 2).

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 interaction-pair complex or a heterodimeric action-pair 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 protein complexes comprising at least one TGF-beta superfamily type I or type II receptor polypeptide 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 single-arm 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: 208). Dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants. In part, the disclosure provides polypeptides comprising amino acid sequences with 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 208. Naturally occurring variants in G1Fc would include E134D and M136L according to the numbering system used in SEQ ID NO: 208 (see Uniprot P01857).

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10      1  THTCPPCPAP  ELLGGPSVFL  FPPKPKDTLM  ISRTPEVTCV  VVDVSHEDPE
      51  VKFNWYVDGV  EVHNAKTKPR  EEQYNSTYRV  VSVLTVLHQD  WLNGKEYKCK
     101  VSNKALPAPI  EKTISKAKGQ  PREPQVYTLF  PSREEMTKNQ  VSLTCLVKGF
     151  YPSDIAVEWE  SNGQPENNYK  TPPVLDSDG  SFFLYSKLTV  DKSRWQQGNV
     201  FSCSVMEAL  HNHYTQKSL  LSPGK      (SEQ ID NO: 208)

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15 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: 209). Dotted underline indicates the hinge region and double underline indicates positions where there are database conflicts in the sequence (according to UniProt P01859). In part, the disclosure provides polypeptides comprising amino acid sequences with 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 209.

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20      1  VECPPCPAPP  VAGPSVFLFP  PKPKDTLMIS  RTPEVTCVVV  DVSHEDPEVQ
      51  FNWYVDGVEV  HNAKTKPREE  QFNSTFRVVS  VLTVVHQDWL  NGKEYKCKVS
     101  NKGLPAPIEK  TISKTKGQPR  EPQVYTLPPS  REEMTKNQVS  LTCLVKGFYP
     151  SDIAVEWESN  GQPENNYKTT  PPMLDSGSGF  FLYSKLTVDK  SRWQQGNVFS
     25  201  CSVMHEALHN  HTQKSLSL  PGK      (SEQ ID NO: 209)

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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: 210) contains a short hinge region consisting of a single 15-residue segment, whereas the second G3Fc sequence (SEQ ID NO: 211) 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 amino acid sequences with 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NOs: 210 and 211.

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1  EPKSCDTPPP CPRCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
51 VSHEDPEVQF KQYVDGVEVH NAKTKPREEQ YNSTFRVSV LTVLHQDWLN
5  101 GKEYKCKVSN KALPAPIEKT ISKTGQPRE PQVYTLPPSR EEMTKNQVSL
151 TCLVKGFYPS DIAVEWESSG QPENNYNTTP PMLDSDGSFF LYSKLTVDKS
201 RWQQGNIFSC SVMHEALHNR FTQKSLSLSP GK (SEQ ID NO: 210)

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1  ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
10 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: 211)

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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: 210, 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: 212). Dotted underline indicates the hinge region. In part, the disclosure provides polypeptides comprising amino acid sequences with 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 212.

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1  ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ
51 EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE
101 YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL
151 VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ

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201 EGNVFSCSVM HEALHNHYTQ KSLSLSLGK (SEQ ID NO: 212)

A variety of engineered mutations in the Fc domain are presented herein with respect to the G1Fc sequence (SEQ ID NO: 208), 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: 208, 209, 210, and 212. 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: 208, 209, 210, 211, or 212) 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: 208), the human IgG1 heavy chain constant domain (Uniprot P01857), and the human IgG1 heavy chain is as follows.

Correspondence of C _H 3 Positions in Different Numbering Systems		
G1Fc (Numbering begins at first threonine in hinge region)	IgG1 heavy chain constant domain (Numbering begins at C _H 1)	IgG1 heavy chain (EU numbering scheme of Kabat et al., 1991*)
Y127	Y232	Y349
S132	S237	S354
E134	E239	E356
T144	T249	T366
L146	L251	L368
K170	K275	K392
D177	D282	D399
Y185	Y290	Y407
K187	K292	K409
* Kabat et al. (eds) 1991; pp. 688-696 in <i>Sequences of Proteins of Immunological Interest</i> , 5 th 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]. 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.

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 be 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 receptor single-arm 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

5 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 PREPQVYTLPSRKEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLKSDG SFFLYSKLTV DKSRWQQGNV
10  201 FSCSVMEAL HNHYTQKSLS LSPGK (SEQ ID NO: 200)

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1   THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTLPSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYD TTPPVLDSDG SFFLYSDLTV DKSRWQQGNV
15  201 FSCSVMEAL HNHYTQKSLS LSPGK (SEQ ID NO: 201)

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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

20 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 receptor single-arm heteromeric complex). In this

25 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

30 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 PREPQVYTL PSREEMTKNQ VSLYCLVKGF
 151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
 5 201 FSCSVMEAL HNHYTQKSLS LSPGK (SEQ ID NO: 202)

1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
 101 VSNKALPAPI EKTISKAKGQ PREPQVYTL PSREEMTKNQ VSLTCLVKGF
 151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLTSKLTV DKSRWQQGNV
 10 201 FSCSVMEAL 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 PREPQVYTL PCREEMTKNQ VSLWCLVKGF
 151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
 25 201 FSCSVMEAL HNHYTQKSLS LSPGK (SEQ ID NO: 204)

1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
 101 VSNKALPAPI EKTISKAKGQ PREPQVCTL PSREEMTKNQ VSLSCAVKGF
 151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSKLTV DKSRWQQGNV
 30 201 FSCSVMEAL 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 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. 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 receptor 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).

```

15      1  THTCPPCPAP  ELLGGPSVFL  FPPKPKDTLM  ISRTPEVTCV  VVDVSHEDPE
      51  VKFNWYVDGV  EVHNAKTKPR  EEQYNSTYRV  VSVLTVLHQD  WLNGKEYKCK
     101  VSNKALPAPI  EKTISKAKGQ  PFRPEVHLLP  PSREEMTKNQ  VSLTCLARGF
     151  YPKDIAVEWE  SNGQPENNYK  TTPSRQEPSQ  GTTTFFAVTSK  LTVDKSRWQQ
     201  GNVFSCSVMH  EALHNHYTQK  TISLSPGK      (SEQ ID NO: 206)

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20      1  THTCPPCPAP  ELLGGPSVFL  FPPKPKDTLM  ISRTPEVTCV  VVDVSHEDPE
      51  VKFNWYVDGV  EVHNAKTKPR  EEQYNSTYRV  VSVLTVLHQD  WLNGKEYKCK
     101  VSNKALPAPI  EKTISKAKGQ  PREPQVYTL  PPSEELALNE  LVTLTCLVKG
     151  FYPSDIAVEW  ESNGQELPRE  KYLTWAPVLD  SDGSFFLYSI  LRVAAEDWKK
     201  GDTFSCSVMH  EALHNHYTQK  SLDRSPGK      (SEQ ID NO: 207)

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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 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 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 receptor 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).

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1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
15 101 VSNKALPAPI EKTISKAKGQ PREPQVYTL P SREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMEAL HNHYTQKSLS LSPGKGGSAQ LEKELQALEK ENAQLEWELQ
251 ALEKELAQGA T (SEQ ID NO: 213)

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1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
20 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTL P SREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMEAL HNHYTQKSLS LSPGKGGSAQ LKKKLQALKK KNAQLKWKLO
251 ALKKKLAQGA T (SEQ ID NO: 214)

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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 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 or type II receptor polypeptide domain. The TGF-beta superfamily type I 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 or type II receptor fusion polypeptide 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 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), TG₄ (SEQ ID NO: 60), SG₄ (SEQ ID NO: 61), TG₃ (SEQ ID NO: 62), or SG₃ (SEQ ID NO: 63) singlets, or repeats. In certain embodiments, a TGF-beta superfamily type I or type II receptor fusion polypeptide 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 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 or type II receptor fusion polypeptide 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 or type II receptor polypeptide domain, and C is an immunoglobulin Fc domain.

Preferred fusion polypeptides comprise the amino acid sequence set forth in any one of SEQ ID NOs: 101, 103, 104, 106, 107, 109, 110, 112, 113, 115, 116, 118, 119, 121, 122, 124, 125, 127, 128, 130, 131, 133, 134, 136, and 401-424.

In some embodiments, TGF-beta superfamily receptor single-arm heteromultimer complexes 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 QIAexpressTM system (Qiagen) useful with (HIS₆) 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 contain 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 polypeptides (including, for example, fusion polypeptides comprising a TGF-beta superfamily type I 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 polypeptides, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous moiety, such as polyethylene glycol.

In preferred embodiments, TGF-beta superfamily receptor single-arm heteromultimer complexes 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 single-arm 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 certain embodiments, TGF-beta superfamily type I or type II receptor polypeptides, as well as single-arm heteromultimer complexes 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 TGF β superfamily type I 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.

3. Nucleic Acids Encoding TGF β Superfamily Receptor Polypeptides

In certain embodiments, the present disclosure provides isolated and/or recombinant nucleic acids encoding TGF β superfamily type I 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 processed 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 single-arm 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 TGF β superfamily type I 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, 102, 105, 108, 114, 117, 120, 123, 126, 129, 132, 135, 303, 304, 307, 308, 311, and 312. 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, 102, 105, 108, 114, 117, 120, 123, 126, 129, 132, 135, 303, 304, 307, 308, 311, and 312.

In certain embodiments, TGF β superfamily type I 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%, 97%, 98%, or 99% 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, 102, 105, 108, 114, 117, 120, 123, 126, 129, 132, 135, 303, 304, 307, 308, 311, and 312. One of ordinary skill in the art will appreciate that nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 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, 102, 105, 108, 114, 117, 120, 123, 126, 129, 132, 135, 303, 304, 307, 308, 311, and 312 are 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, 102, 105, 108, 114, 117, 120, 123, 126, 129, 132, 135, 303, 304, 307, 308, 311, and 312, 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, 102, 105, 108, 114, 117, 120, 123, 126, 129, 132, 135, 303, 304, 307, 308, 311, and 312, 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, 102, 105, 108, 114, 117, 120, 123, 126, 129, 132, 135, 303, 304, 307, 308, 311, and 312 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 TGF β superfamily type I 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 TGF β superfamily type I 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 TGF β superfamily type I or type II receptor polypeptide. 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 TGF β superfamily type I 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.*

5 *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

10 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

15 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

20 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 β -gal

25 containing pBlueBac III).

In a preferred embodiment, a vector will be designed for production of the subject TGF β superfamily type I or type II receptor polypeptide 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

30 be used to cause expression of the subject TGF β superfamily type I 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 TGF β superfamily type I or type II receptor polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a TGF β superfamily type I 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 TGF β superfamily type I or type II receptor polypeptides. For example, a host cell transfected with an expression vector encoding a TGF β superfamily type I or type II receptor polypeptide can be cultured under appropriate conditions to allow expression of the TGF β superfamily type I 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 TGF β superfamily type I 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 TGF β superfamily type I or type II receptor polypeptides and affinity purification with an agent that binds to a domain fused to TGF β superfamily type I or type II receptor polypeptide (e.g., a protein A column may be used to purify a TGF β superfamily type I receptor-Fc or type II receptor-Fc fusion polypeptide or protein complex). In some embodiments, the TGF β superfamily type I or type II receptor polypeptide is a fusion polypeptide or protein complex 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 TGF β superfamily type I receptor-Fc or type II receptor-Fc fusion polypeptide or protein complex 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 TGF β superfamily type I or type II receptor polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni²⁺ metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified TGF β superfamily type I or type II receptor polypeptide or protein complex. *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 TGF β superfamily type I and type II receptor single-arm heteromultimer complexes to identify compounds (agents) which are agonists or antagonists of TGF β 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 TGF β 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 TGF β 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 receptor single-arm heteromultimer complex to its binding partner, such as a TGF β 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- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, GDNF, neurturin, artemin, persephin, MIS, and Lefty). Alternatively, the assay can be used to identify compounds that enhance binding of a TGF-beta superfamily receptor single-arm heteromultimer complex to its binding partner such as an TGF β superfamily ligand. In a further embodiment, the compounds can be identified by their ability to interact with a TGF-beta superfamily receptor single-arm heteromultimer complex 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, digoxigenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S-transferase (GST), photoactivatable crosslinkers or any combinations thereof.

In many drug-screening programs which test libraries of compounds and natural
 5 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.

10 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 receptor single-arm heteromultimer complex and its binding partner (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7,
 15 BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, GDNF, neurturin, artemin, persephin, MIS, and Lefty).

Merely to illustrate, in an exemplary screening assay of the present disclosure, the
 20 compound of interest is contacted with an isolated and purified TGF-beta superfamily receptor single-arm heteromultimer complex 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 receptor single-arm heteromultimer complex is then added the appropriate TGF-beta superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4,
 25 BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, GDNF, neurturin, artemin, persephin, MIS, and Lefty). Detection and quantification of complexes between single-arm heteromultimers and
 30 superfamily ligands provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the TGF-beta superfamily receptor single-arm heteromultimer complex 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 receptor single-arm heteromultimer complex, 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 receptor single-arm heteromultimer complex 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 ^3H), fluorescently labeled (*e.g.*, FITC), or enzymatically labeled TGF-beta superfamily receptor single-arm heteromultimer complex and 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 receptor single-arm heteromultimer complex 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 receptor single-arm heteromultimer complex 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 receptor single-arm heteromultimer complex 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 receptor single-arm heteromultimer complex of the disclosure. The interaction between the compound and the TGF-beta superfamily receptor single-arm heteromultimer complex 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 receptor single-arm heteromultimer complex. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding a TGF-beta superfamily receptor single-arm heteromultimer complex 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, of the present disclosure can be used to treat or prevent a disease or condition that is associated with abnormal activity of a TGF β superfamily receptor (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, ActRIIB, BMPRII, TGFBRII, and MISRII) and/or a TGF β 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- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, GDNF, neurturin, artemin, persephin, MIS, and Lefty). These diseases, disorders or conditions are generally referred to herein as "TGF β superfamily-associated conditions." 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, as described herein. The terms "subject," an "individual," or a "patient" are interchangeable throughout the specification. Any of the TGF-beta superfamily receptor single-arm

5 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
10 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
15 discerned in the diagnosis provided by a physician or other health care provider and the intended result of administration of the therapeutic agent.

Native 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,
20 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.

25 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-
30 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm 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 receptor single-arm heteromultimer complexes of the disclosure may act as

GDF8 inhibitors (antagonists), and constitute an alternative means of blocking signaling by GDF8 and/or related TGF β superfamily ligands *in vivo* in DMD and BMD patients.

Similarly, TGF-beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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
5 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
10 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 receptor single-arm heteromultimer complex pharmaceutical compositions may be beneficially used to prevent, treat, or alleviate the symptoms of the cachexia syndrome, where muscle growth is desired.

15 In certain embodiments, a TGF-beta superfamily receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor
20 single-arm 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, of the present disclosure may find medical utility in the healing of bone fractures
25 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,
30 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer complexes of the disclosure may also be useful in the treatment of osteoporosis. Further, TGF-beta superfamily receptor single-arm heteromultimer complexes may be used in repair of cartilage defects and prevention/reversal of osteoarthritis.

Rosen et al. (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 7th ed. American Society for Bone and Mineral Research, Washington D.C. (incorporated herein by reference) provides an extensive discussion of bone disorders that may be subject to treatment with a TGF-beta superfamily receptor single-arm heteromultimer complex or with combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes. A partial listing is provided herein. 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 receptor single-arm heteromultimer complexes of the disclosure in admixture with a pharmaceutically acceptable vehicle, carrier, or matrix.

In some embodiments, a TGF-beta superfamily receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 produced 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 complex. 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer complex, or a combination of complexes, 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 receptor single-arm heteromultimer complex 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, in a patient.

In another embodiment, a TGF-beta superfamily receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, may be used in patients with chronic kidney disease mineral bone disorder (CKD-MBD), a broad syndrome of interrelated skeletal, cardiovascular, and mineral-metabolic disorders arising from kidney disease. CKD-MBD encompasses various skeletal pathologies often referred to as renal osteodystrophy (ROD), which is a preferred embodiment for treatment with a TGF-beta superfamily receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes. Depending on the relative contribution of different pathogenic factors, ROD is manifested as diverse pathologic patterns of bone remodeling (Hruska et al., 2008, Chronic kidney disease mineral bone disorder (CKD-MBD); in Rosen et al. (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 7th ed. American Society for Bone and Mineral Research, Washington D.C., pp 343-349). At one end of the spectrum is ROD with uremic osteodystrophy and low bone turnover, characterized by a low number of active remodeling sites, profoundly suppressed bone formation, and low bone resorption. At the other extreme is ROD with hyperparathyroidism, high bone turnover, and osteitis fibrosa. Given that a TGF-beta superfamily receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, may

exert both anabolic and antiresorptive effects, these agents may be useful in patients across the ROD pathology spectrum.

A TGF-beta superfamily receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer complexes may be particularly advantageous if administered with other bone-active agents. A patient may benefit from conjointly receiving a TGF-beta superfamily receptor single-arm heteromultimer complex and taking calcium supplements, vitamin D, appropriate exercise and/or, in some cases, other medication. Examples of other medications include, 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
5 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 receptor
10 single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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
15 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
20 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
25 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
30 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, of the disclosure.

In some embodiments, a TGF-beta superfamily receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer complex, or combinations of TGF-beta superfamily receptor single-arm heteromultimer complexes, of the disclosure.

In certain aspects, a TGF-beta superfamily receptor single-arm heteromultimer complex, or a combination of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer

complex, or a combination of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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, a TGF-beta superfamily receptor single-arm heteromultimer complex, or a combination of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer complex, or a combination of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer complex, or a combination of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer complex, or a combination of TGF-beta superfamily receptor single-arm heteromultimer complexes, 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 receptor single-arm heteromultimer complex, or a combination of TGF-beta superfamily receptor single-arm heteromultimer complexes, to increase red blood cell levels, and then blood is drawn and stored for later use in transfusions.

One or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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 erythematosus (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].

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 receptor single-arm heteromultimer complexes 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 receptor single-arm 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 receptor single-arm 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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

5 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 receptor single-arm heteromultimer complexes 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-
10 beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes of the disclosure and one or more additional therapeutic agents for treating MDS including, for example, thalidomide, lenalidomide,
15 azacitadine, decitabine, erythropoietins, deferoxamine, antithymocyte globulin, and filgrastim (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
20 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
25 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
30 superfamily receptor single-arm 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 receptor

single-arm heteromultimer complexes 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. 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 receptor single-arm heteromultimer complexes of the disclosure, optionally combined with an EPO receptor activator, can be used to treat or prevent a
5 thalassemia syndrome.

In some embodiments, one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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);

10 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
15 (congenital dyserythropoietic anemia, refractory megaloblastic anemia, or erythroleukemia); myelophthistic anemias including, for example, myelofibrosis (myeloid metaplasia) and myelophthisis; congenital erythropoietic porphyria; and lead poisoning.

In certain embodiments, one or more TGF-beta superfamily receptor single-arm heteromultimer complexes of the disclosure may be used in combination with supportive
20 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
25 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
30 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
5 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.*
10 (2010) Ann NY Acad Sci 1202:79-86].

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
15 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 receptor single-arm heteromultimer complexes of the disclosure can be
20 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.

25 In certain embodiments, one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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
30 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 receptor single-arm heteromultimer complexes of the disclosure, optionally combined with an EPO receptor activator, would also be
5 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
10 therapeutically effective amount of one or more TGF-beta superfamily receptor single-arm heteromultimer complexes of the disclosure and a EPO receptor activator. In certain embodiments, one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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
15 of EPO. These methods may be used for therapeutic and prophylactic treatments of a patient.

One or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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
20 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,
25 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 receptor single-arm heteromultimer complexes of
30 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 receptor single-arm heteromultimer complex

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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes of the disclosure, then onset of administration of the one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes of the disclosure. Alternatively, a therapeutic regimen may be developed for the patient that combines one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 heteromultimer complexes of the disclosure. A patient's baseline values for one or more hematologic parameters prior to treatment with one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes of the disclosure or additional dosing with another therapeutic agent. For example, if administration of one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes of the disclosure may be reduced in amount or frequency in order to decrease the effects of the one or more TGF-beta superfamily receptor single-arm heteromultimer complexes of the disclosure on the one or more hematologic parameters. If administration of one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes of the disclosure has elevated blood pressure, then dosing with the one or more TGF-beta superfamily receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 receptor single-arm heteromultimer complexes 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 general, 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
5 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
10 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
15 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
20 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
25 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.

30 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 the 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 TGF β superfamily receptor single-arm heteromultimer complexes 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, guar, 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, methacrylic 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 receptor single-arm heteromultimer complex 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 of the present invention, and are not intended to limit the invention.

5 **Example 1. Generation and characterization of a single-arm ActRIIB-Fc heterodimer**

Applicants constructed a soluble single-arm ActRIIB-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human ActRIIB was fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain.

10 The individual constructs are referred to as monomeric Fc polypeptide and ActRIIB-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

A methodology for promoting formation of ActRIIB-Fc:Fc heteromeric complexes rather than ActRIIB-Fc:ActRIIB-Fc or Fc:Fc homodimeric complexes is to introduce alterations in the amino acid sequence of the Fc domains to guide the formation of
15 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 monomeric Fc polypeptide sequences of SEQ ID NOs: 104-106 and 137-139, respectively, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to
20 introduce anionic amino acids at the interaction face. The ActRIIB-Fc fusion polypeptide and monomeric Fc polypeptide each employ the tissue plasminogen activator (TPA) leader: MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 100).

The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 104) is shown below:

1 MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
25 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
30 301 EWESNGQPEN NYKTTTPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQK SLSLSPGK (SEQ ID NO: 104)

The leader (signal) sequence and linker are underlined. To promote formation of the ActRIIB-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ActRIIB-Fc:ActRIIB-Fc or Fc:Fc), 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: 104 may optionally be provided with the C-terminal lysine (K) removed.

This ActRIIB-Fc fusion polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 105):

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1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
10  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
15  301  GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
    351  GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
    401  CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
    451  CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCAAA
    501  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
20  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 CTCCAAGCC AAAGGGCAGC CCCGAGAACC
25  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 GGAACGTCT TCTCATGCTC CGTGATGCAT
30  1051  GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
    1101  TAAA      (SEQ ID NO: 105)

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The mature ActRIIB-Fc fusion polypeptide (SEQ ID NO: 106) is as follows and may optionally be provided with the C-terminal lysine removed.

1 GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51 IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
 101 GGPEVTYIEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
 151 RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
 5 201 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
 251 RKEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLKSDGSF
 301 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL S PGK
 (SEQ ID NO: 106)

The complementary human G1Fc polypeptide (SEQ ID NO: 137) employs the TPA
 10 leader and is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGASNTKVD KRVTGGGTHT CPPCPAPELL
 51 GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH
 101 NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
 151 ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG
 15 201 QPENNYDTTP PVLDSDGSFF LYSDLTVDKS RWQQGNVFSC SVMHEALHNH
 251 YTQKSLSLSP GK (SEQ ID NO: 137)

The leader sequence is underlined, and an optional N-terminal extension of the Fc
 polypeptide is indicated by double underline. To promote formation of the ActRIIB-Fc:Fc
 heterodimer rather than either of the possible homodimeric complexes, two amino acid
 20 substitutions (replacing lysines with anionic residues) can be introduced into the monomeric
 Fc polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID
 NO: 137 may optionally be provided with the C-terminal lysine removed.

This complementary Fc polypeptide is encoded by the following nucleic acid (SEQ
 ID NO: 138).

25 1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51 AGTCTTCGTT TCGCCCGGCG CCAGCAACAC CAAGGTGGAC AAGAGAGTTA
 101 CCGGTGGTGG AACTCACACA TGCCACCGT GCCCAGCACC TGAATCCTG
 151 GGGGGACCGT CAGTCTTCCT CTTCCCCCA AAACCAAGG ACACCCTCAT
 201 GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG
 30 251 AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT
 301 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT
 351 GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT

401 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC
 451 ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
 501 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG
 551 TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG
 5 601 CAGCCGGAGA ACAACTACGA CACCACGCCT CCCGTGCTGG ACTCCGACGG
 651 CTCCTTCTTC CTCTATAGCG ACCTCACCGT GGACAAGAGC AGGTGGCAGC
 701 AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC
 751 TACACGCAGA AGAGCCTCTC CCTGTCTCCG GTAAA

(SEQ ID NO: 138)

10 The sequence of the mature monomeric Fc polypeptide is as follows (SEQ ID NO: 139) and may optionally be provided with the C-terminal lysine removed.

1 SNTKVDKRV T GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV
 51 TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL
 101 HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT
 15 151 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYDTTPPVLD SDGSFFLYSD
 201 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK

(SEQ ID NO: 139)

The ActRIIB-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 106 and SEQ ID NO: 139, respectively, may be co-expressed and purified from a CHO cell
 20 line to give rise to a single-arm heteromeric protein complex comprising ActRIIB-Fc:Fc.

In another approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond, as illustrated in the ActRIIB-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 403-404 and 425-426,
 25 respectively.

The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 403) employs the TPA leader and is shown below:

1 MDAMKRG LCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQ S
 51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
 30 101 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 NYKTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
 351 EALHNHYTQK SLSLSPGK (SEQ ID NO: 403)

The leader sequence and linker are underlined. To promote formation of the ActRIIB-Fc: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 tryptophan) 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: 403 may optionally be provided with the C-terminal lysine removed.

10 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
 15 201 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC
 251 REEMTKNQVS LWCLVKGFYP SDIAVEWESN GPENNYKTT PPVLDSGGSF
 301 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
 (SEQ ID NO: 404)

The complementary form of monomeric Fc polypeptide (SEQ ID NO: 425) uses the TPA leader and is as follows.

1 MDAMKRGLCC VLLLCGAVFV SPGASNTKVD KRVTGGGTHT CPPCPAPELL
 51 GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH
 101 NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
 151 ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL SCAVKGFYPS DIAVEWESNG
 25 201 QPENNYKTTT PVLDSGGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH
 251 YTQKSLSLSP GK (SEQ ID NO: 425)

The leader sequence is underlined, and an optional N-terminal extension of the Fc polypeptide is indicated by double underline. To promote formation of the ActRIIB-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, four amino acid substitutions can be introduced into the monomeric Fc polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 425 may optionally be provided with the C-terminal lysine removed.

The mature monomeric Fc polypeptide sequence (SEQ ID NO: 426) is as follows and may optionally be provided with the C-terminal lysine removed.

```

1  SNTKVDKRV T GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV
51  TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL
5  101  HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVC TLPPSREEMT
151  KNQVSLSCAV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLVSK
201  LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
    (SEQ ID NO: 426)

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The ActRIIB-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 404 and SEQ ID NO: 426, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ActRIIB-Fc:Fc.

Purification of various ActRIIB-Fc: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.

A BiacoreTM-based binding assay was used to compare ligand binding selectivity of the single-arm ActRIIB-Fc heterodimeric complex described above with that of ActRIIB-Fc homodimeric complex. Single-arm ActRIIB-Fc and homodimeric ActRIIB-Fc 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) typically associated with the most effective ligand traps are denoted by gray shading.

Ligand binding by single-arm ActRIIB-Fc compared to ActRIIB-Fc homodimer						
Ligand	ActRIIB-Fc homodimer			Single-arm ActRIIB-Fc		
	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	3.0×10^7	3.0×10^{-3}	99
Activin B	5.1×10^6	1.0×10^{-4}	20	3.5×10^6	4.2×10^{-4}	120
BMP6	3.2×10^7	6.8×10^{-3}	210	4.2×10^7	2.9×10^{-2}	690
BMP9	1.4×10^7	1.1×10^{-3}	78	No binding		

BMP10	2.3×10^7	2.6×10^{-4}	11	8.0×10^7	9.7×10^{-3}	120
GDF3	1.4×10^6	2.2×10^{-3}	1500	1.1×10^6	1.3×10^{-2}	12000
GDF8	8.3×10^5	2.3×10^{-4}	280	3.5×10^6	1.0×10^{-3}	290
GDF11	5.0×10^7	1.1×10^{-4}	2	3.6×10^7	7.2×10^{-4}	20

These comparative binding data demonstrate that single-arm ActRIIB-Fc has greater ligand selectivity than homodimeric ActRIIB-Fc. Whereas ActRIIB-Fc homodimer binds strongly to five important ligands (see cluster of activin A, activin B, BMP10, GDF8, and GDF11 in Fig. 6), single-arm ActRIIB-Fc discriminates more readily among these ligands. Thus, single-arm ActRIIB-Fc binds strongly to activin B and GDF11 and with intermediate strength to GDF8 and activin A. In further contrast to ActRIIB-Fc homodimer, single-arm ActRIIB-Fc displays only weak binding to BMP10 and no binding to BMP9. See Figure 6.

These results indicate that single-arm ActRIIB-Fc is a more selective antagonist than ActRIIB-Fc homodimer. Accordingly, single-arm ActRIIB-Fc will be more useful than 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, GDF8, and GDF11 but minimize antagonism of one or more of BMP9, BMP10, BMP6, and GDF3. Selective inhibition of ligands in the former group would be particularly advantageous therapeutically because they constitute a subfamily which tends to differ functionally from the latter group and its associated set of clinical conditions.

Example 2. Generation and characterization of a single-arm ALK3-Fc heterodimer

Applicants constructed a soluble single-arm ALK3-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human ALK3 was fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and ALK3-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

Formation of a single-arm ALK3-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first

approach, illustrated in the ALK3-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 122-124 and 140-142, 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.

5 The ALK3-Fc fusion polypeptide employs the TPA leader and is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51 LAPEDTL PFL KCYCSGHCPD DAINNTCITN GHCF AIIEED DQGETTLASG
 101 CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
 151 SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 10 201 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 251 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
 301 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDS DGSFF LYSDLTVDKS
 351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G (SEQ ID NO: 122)

The leader and linker sequences are underlined. To promote formation of the ALK3-
 15 Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ALK3-
 Fc:ALK3-Fc or Fc:Fc, two amino acid substitutions (replacing lysines with anionic amino
 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: 122 may optionally be provided
 with a lysine added at the C-terminus.

20 This ALK3-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID
 NO: 123).

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51 AGTCTTCGTT TCGCCCGGCG CCCAGAATCT GGATAGTATG CTT CATGGCA
 101 CTGGGATGAA ATCAGACTCC GACCAGAAAA AGTCAGAAAA TGGAGTAACC
 25 151 TTAGCACCAG AGGATACCTT GCCTTTTTTTA AAGTGCTATT GCTCAGGGCA
 201 CTGTCCAGAT GATGCTATTA ATAACACATG CATAACTAAT GGACATTGCT
 251 TTGCCATCAT AGAAGAAGAT GACCAGGGAG AAACCACATT AGCTTCAGGG
 301 TGTATGAAAT ATGAAGGATC TGATTTTCAG TGCAAAGATT CTCCAAAAGC
 351 CCAGCTACGC CGGACAATAG AATGTTGTCT GACCAATTTA TGTAACCAGT
 30 401 ATTTGCAACC CACACTGCCC CCTGTTGTCA TAGGTCCGTT TTTTGATGGC
 451 AGCATTCGAA CCGGTGGTGG AACTCACACA TGCCACCGT GCCCAGCACC
 501 TGAACCTCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCA 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
 5 751 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
 10 1001 ACTCCGACGG CTCCTTCTTC CTCTATAGCG ACCTCACCGT GGACAAGAGC
 1051 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
 1101 GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGT
 (SEQ ID NO: 123)

The mature ALK3-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 124)
 15 and may optionally be provided with a lysine added at the C-terminus.

1 GAQNLDMLH GTGMKSDSDQ KSENGVTLA PEDTLPFLKC YCSGHCPDDA
 51 INNTCITNGH CFAIIEEDDQ GETTLASGCM KYEGSDFQCK DSPKAQLRRT
 101 IECCRTNLCN QYLQPTLPPV VIGPFFDGS I RTGGGTHTCP PCPAPELLGG
 151 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
 20 201 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
 251 KAKGQPREPQ VYTLPPSREE MTKNQVSLTCLVKGFYPSDI AVEWESNGQP
 301 ENNYDTTPPV LDSDGSFFLY SCLTVDKSRW QQGNVFSCSV MHEALHNHYT
 351 QKSLSLSPG (SEQ ID NO: 124)

The complementary human G1Fc polypeptide (SEQ ID NO: 140) employs the TPA
 25 leader and is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGASNTKVD KRVTGGGTHT CP~~PC~~PAPELL
 51 GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH
 101 NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
 151 ISKAKGQPRE PQVYTLPPSR KEMTKNQVSL TCLVKGFYPS DIAVEWESNG
 30 201 QPENNYKTTP PVLKSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH
 251 YTQKSLSLSP GK (SEQ ID NO: 140)

The leader sequence is underlined, and an optional N-terminal extension of the Fc polypeptide is indicated by double underline. To promote formation of the ALK3-Fc:Fc

heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the monomeric Fc polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 140 may optionally be provided with the C-terminal lysine removed.

5 This complementary Fc polypeptide is encoded by the following nucleic acid (SEQ ID NO: 141).

```

1   ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51  AGTCTTCGTT TCGCCCGGCG CCAGCAACAC CAAGGTGGAC AAGAGAGTTA
101 CCGGTGGTGG AACTCACACA TGCCCACCGT GCCCAGCACC TGAATCCTG
10  151 GGGGGACCGT CAGTCTTCCT CTTCCCCCA AAACCCAAGG ACACCCTCAT
201 GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG
251 AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT
301 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT
351 GGTCAAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT
15  401 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC
451 ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
501 CCCATCCCGG AAGGAGATGA CCAAGAACCA GGTCAAGCCTG ACCTGCCTGG
551 TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG
601 CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGA AGTCCGACGG
20  651 CTCCTTCTTC CTCTATAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
701 AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC
751 TACACGCAGA AGAGCCTCTC CCTGTCTCCG GTAAA
      (SEQ ID NO: 141)

```

25 The sequence of the mature monomeric Fc polypeptide is as follows (SEQ ID NO: 142) and may optionally be provided with the C-terminal lysine removed.

```

1   SNTKVDKRV T GGGTHTCP PC PAPELLGG PS VFLFPPK PKD TLMISRTPEV
51  TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL
101 HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRKEMT
151 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPV LK SDGSFFLYSK
30  201 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
      (SEQ ID NO: 142)

```

The ALK3-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 124 and SEQ ID NO: 142, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK3-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ALK3-Fc and Fc polypeptide sequences of SEQ ID NOs: 415-416 and 427-428, respectively.

The ALK3-Fc fusion polypeptide (SEQ ID NO: 415) uses the TPA leader and is as follows:

```

10      1  MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
      51  LAPEDTLPLF KYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
     101  CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
     151  SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
     201  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
     15  251  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPSPR EEMTKNQVSL
     301  SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSGGSFF LVSKLTVDKS
     351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 415)

```

The leader sequence and linker are underlined. To promote formation of the ALK3-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, 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: 415 may optionally be provided with the C-terminal lysine removed.

The mature ALK3-Fc fusion polypeptide (SEQ ID NO: 416) is as follows and may optionally be provided with the C-terminal lysine removed.

```

25      1  GAQNLDSMLH GTGMKSDSDQ KKSENGVTLA PEDTLPLFLKC 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
     30  251  KAKGQPREPQ VCTLPPSREE MTKNQVSLSC AVKGFYPSDI AVEWESNGQP
     301  ENNYKTTPPV LDSDGSFFLV SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
     351  QKSLSLSPGK (SEQ ID NO: 416)

```

The complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 427) employs the TPA leader and is as follows:

```

1  MDAMKRG LCC VLLLCGAVFV SPGASNTKVD KRVTGGGTHT CPPCPAPELL
51 GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NQYVDGVEVH
5  101 NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
151 ISKAKGQPRE PQVYTLPPCR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG
201 QPENNYKTP PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNNH
251 YTQKSLSLSP GK (SEQ ID NO: 427)

```

The leader sequence is underlined, and an optional N-terminal extension of the Fc polypeptide is indicated by double underline. To promote formation of the ALK3-Fc: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 tryptophan) can be introduced into the monomeric Fc polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 427 may optionally be provided with the C-terminal lysine removed.

The sequence of the mature monomeric Fc polypeptide is as follows (SEQ ID NO: 428) and may optionally be provided with the C-terminal lysine removed.

```

1  SNTKVDKRV T GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV
51 TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL
20 101 HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPCREEMT
151 KNQVSLWCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK
201 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
(SEQ ID NO: 428)

```

The ALK3-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 416 and SEQ ID NO: 428, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric complex comprising ALK3-Fc:Fc.

Purification of various ALK3-Fc: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.

A Biacore™-based binding assay was used to compare ligand binding selectivity of the single-arm ALK3-Fc heterodimeric complex described above with that of an ALK3-Fc homodimeric complex. The single-arm ALK3-Fc and homodimeric ALK3-Fc 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) typically associated with the most effective ligand traps are denoted by gray shading.

Ligand binding of single-arm ALK3-Fc compared to ALK3-Fc homodimer						
Ligand	ALK3-Fc homodimer			Single-arm ALK3-Fc		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	No binding			No binding		
Activin B	No binding			No binding		
Activin AB	No binding			No binding		
Activin AC	No binding			No binding		
BMP2	6.8×10^5	8.9×10^{-5}	130	7.9×10^5	2.5×10^{-4}	310
BMP4	3.0×10^5	5.3×10^{-5}	178	4.9×10^5	4.6×10^{-5}	93
BMP5	2.9×10^4	2.0×10^{-3}	70000	1.2×10^5	5.3×10^{-3}	45000
BMP6	1.4×10^5	4.9×10^{-3}	35000	No binding		
BMP7	1.2×10^6	1.8×10^{-2}	15000	No binding		
BMP10	No binding			No binding		
GDF5	4.8×10^5	1.1×10^{-2}	22000	No binding		
GDF6	3.4×10^4	1.3×10^{-3}	40000	No binding		
GDF7	2.2×10^5	2.7×10^{-3}	12000	4.6×10^5	1.0×10^{-2}	22000
GDF8	No binding			No binding		
GDF11	No binding			No binding		

These comparative data indicate that single-arm ALK3-Fc has greater ligand selectivity than homodimeric ALK3-Fc. Whereas single-arm ALK3-Fc heterodimer retains the exceptionally tight binding to BMP4 observed with ALK3-Fc homodimer, it exhibits reduced strength of binding to BMP2 and therefore discriminates better between BMP4 and

BMP2 (still a strong binder) than does ALK3-Fc homodimer. Single-arm ALK3-Fc also discriminates better among BMP5 (intermediate binding), GDF7 (weak binding), and GDF6 (no binding) compared to ALK3-Fc homodimer, which binds these three ligands with very similar strength (all intermediate). See Figure 7. Unlike constructs disclosed in Example 1, neither single-arm ALK3-Fc nor homodimeric ALK3-Fc binds activins, GDF8, GDF11, or BMP10.

These results therefore indicate that single-arm ALK3-Fc is a more selective antagonist of BMP4 than is ALK3-Fc homodimer. Single-arm ALK3-Fc can be expected to antagonize BMP4 in a more targeted manner – with reduced effects from concurrent antagonism of BMP2 or BMP5 and especially GDF6 or GDF7 – compared to ALK3-Fc homodimer. Accordingly, single-arm ALK3-Fc will be more useful than ALK3-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 BMP4, BMP2, and potentially BMP5 but minimize antagonism of one or more of BMP6, GDF6, and GDF7.

Example 3. Generation and characterization of a single-arm ActRIIA-Fc heterodimer

Applicants constructed a soluble single-arm ActRIIA-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human ActRIIA was fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and ActRIIA-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

Formation of a single-arm ActRIIA-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the ActRIIA-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 101-103 and 137-139, 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 ActRIIA-Fc fusion polypeptide employs the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
51 GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTPDCVEKK

```

101 DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
 151 CPAPPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
 201 VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
 251 PAPIEKTISK AKGQPREPQV YTLPPSRKEM TKNQVSLTCL VKGFYPSDIA
 5 301 VEWESNGQPE NNYKTTPPVL KSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
 351 HEALHNHYTQ KSLSLSPGK (SEQ ID NO: 101)

The leader and linker sequences are underlined. To promote formation of the ActRIIA-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ActRIIA-Fc:ActRIIA-Fc or Fc-Fc), two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the Fc domain of the fusion polypeptide as indicated by
 10 double underline above. The amino acid sequence of SEQ ID NO: 101 may optionally be provided with the C-terminal lysine removed.

This ActRIIA-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 102).

15 1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51 AGTCTTCGTT TCGCCCGGCG CCGCTATACT TGGTAGATCA GAAACTCAGG
 101 AGTGTCTTTT CTTTAATGCT AATTGGGAAA AAGACAGAAC CAATCAAACCT
 151 GGTGTTGAAC CGTGTTATGG TGACAAAGAT AAACGGCGGC ATTGTTTTTGC
 201 TACCTGGAAG AATATTTCTG GTTCCATTGA AATAGTGAAA CAAGGTTGTT
 20 251 GGCTGGATGA TATCAACTGC TATGACAGGA CTGATTGTGT AGAAAAAAAAA
 301 GACAGCCCTG AAGTATATTT CTGTTGCTGT GAGGGCAATA TGTGTAATGA
 351 AAAGTTTTCT TATTTTCCGG AGATGGAAGT CACACAGCCC ACTTCAAATC
 401 CAGTTACACC TAAGCCACCC ACCGGTGGTG GAACTCACAC ATGCCACCCG
 451 TGCCCAGCAC CTGAACTCCT GGGGGGACCG TCAGTCTTCC TCTTCCCCCC
 25 501 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
 30 751 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: 102)

5 The mature ActRIIA-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 103) and may optionally be provided with the C-terminal lysine removed.

1 ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51 IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPPEM
 101 EVTQPTSNPV TPKPPTGGGT HTCPCCPAPE LLGGPSVFLF PPKPKDTLMI
 10 151 SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
 201 SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
 251 SRKEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLKSDGS
 301 FFLYSLKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
 (SEQ ID NO: 103)

15 As described in Example 1, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 137) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ActRIIA-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with anionic residues) can be introduced into the monomeric Fc polypeptide. The
 20 amino acid sequence of SEQ ID NO: 137 may optionally be provided without the C-terminal lysine. This complementary Fc polypeptide is encoded by the nucleic acid of SEQ ID NO: 138, and the mature monomeric Fc polypeptide (SEQ ID NO: 139) may optionally be provided with the C-terminal lysine removed.

The ActRIIA-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO:
 25 103 and SEQ ID NO: 139, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ActRIIA-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the
 30 ActRIIA-Fc and Fc polypeptide sequences of SEQ ID NOs: 401-402 and 425-426, respectively.

The ActRIIA-Fc fusion polypeptide (SEQ ID NO: 401) uses the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
51  GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
5  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 QGNVFSCSV
10  351  HEALHNHYTQ KSLSLSPGK (SEQ ID NO: 401)

```

The leader sequence and linker are underlined. To promote formation of the ActRIIA-Fc: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 tryptophan) can be introduced into the Fc domain of the ActRIIA fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 401 may optionally be provided with the C-terminal lysine removed.

The mature ActRIIA-Fc fusion polypeptide (SEQ ID NO: 402) is as follows and may optionally be provided with the C-terminal lysine removed.

```

1  ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWK NISGS
20  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
25  301  FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
      (SEQ ID NO: 402)

```

As described in Example 1, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 425) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ActRIIA-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, four amino acid substitutions can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of

SEQ ID NO: 425 and the mature G1Fc polypeptide (SEQ ID NO: 426) may optionally be provided with the C-terminal lysine removed.

The ActRIIA-Fc fusion polypeptide and monomeric Fc polypeptide 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 single-arm heteromeric protein complex comprising ActRIIA-Fc:Fc.

Purification of various ActRIIA-Fc: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.

A BiacoreTM-based binding assay was used to compare ligand binding selectivity of the single-arm ActRIIA-Fc heterodimeric complex described above with that of an ActRIIA-Fc homodimeric complex. The single-arm ActRIIA-Fc and homodimeric ActRIIA-Fc 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) typically associated with the most effective ligand traps are denoted by gray shading.

Ligand binding of single-arm ActRIIA-Fc compared to ActRIIA-Fc homodimer						
Ligand	ActRIIA-Fc homodimer			Single-arm ActRIIA-Fc		
	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	3.0×10^7	9.0×10^{-4}	30
Activin B	7.9×10^6	2.0×10^{-4}	25	2.9×10^7	1.4×10^{-3}	46
BMP5	4.0×10^6	4.5×10^{-3}	1100	4.8×10^7	5.8×10^{-2}	1200
BMP10	2.9×10^7	2.5×10^{-3}	86	2.3×10^7	5.9×10^{-3}	250
GDF8	1.4×10^7	1.4×10^{-3}	99	4.7×10^6	5.0×10^{-3}	1100
GDF11	2.6×10^7	7.2×10^{-4}	28	4.9×10^7	1.1×10^{-2}	220

These comparative binding data indicate that single-arm ActRIIA-Fc has different ligand selectivity than homodimeric ActRIIA-Fc (and also different than single-arm ActRIIB-Fc or homomeric ActRIIB-Fc – see Example 1). Whereas ActRIIA-Fc homodimer exhibits

preferential binding to activin B combined with strong binding to activin A and GDF11, single-arm ActRIIA-Fc has a reversed preference for activin A over activin B combined with greatly enhanced selectivity for activin A over GDF11 (weak binder). See Figure 8. In addition, single-arm ActRIIA-Fc largely retains the intermediate binding to GDF8 and BMP10 observed with ActRIIA-Fc homodimer.

These results indicate that single-arm ActRIIA-Fc heterodimer is an antagonist with substantially altered ligand selectivity compared to ActRIIA-Fc homodimer. Accordingly, single-arm ActRIIA-Fc will be more useful than ActRIIA-Fc homodimer in certain applications where such antagonism is advantageous. Examples include therapeutic applications where it is desirable to antagonize activin A preferentially over activin B while minimizing antagonism of GDF11.

Together the foregoing examples demonstrate that type I or type II receptor polypeptides, when placed in the context of a single-arm heteromeric protein complex, form novel binding pockets that exhibit altered selectivity relative to either type of homomeric protein complex, allowing the formation of novel protein agents for possible use as therapeutic agents.

Example 4. Generation and characterization of a single-arm BMPRII-Fc heterodimer

Applicants constructed a soluble single-arm BMPRII-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human BMPRII was fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and BMPRII-Fc fusion polypeptide, respectively, and the sequences for each are provided below. Applicants also envision additional single-arm BMPRII-Fc heterodimeric complexes comprising the extracellular domain of BMPRII isoform A (SEQ ID NO: 72).

Formation of a single-arm BMPRII-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the BMPRII-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 107-109 and 137-139, 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 BMPRII-Fc fusion polypeptide employs the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
5  51  HENGTI5LCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYE5ECVVT
101 TTPPSIQNGT YRFCCCSTD10L CNVNFTENFP PPDTT10PLSPP HSFNRDE10TGG
151 GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201 EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251 KVS20NKALPAP IEKTISKAKG QPREPQVYTL PPSR20KEMTKN QVSLTCLVKG
10 301 FYPSDIAVEW ESNGQPENNY K10TPPV10L10KSD GSFFLYSKLT VDKSRWQQGN
351 VFSCSVMHEA LHNHYTQKSL SLSPGK (SEQ ID NO: 107)

```

The leader and linker sequences are underlined. To promote formation of the BMPRII-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (BMPRII-Fc:BMPRII-Fc or Fc:Fc), two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the Fc domain of the fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 107 may optionally be provided with the C-terminal lysine removed.

This BMPRII-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 108).

```

20 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 TGTA20AAACAA GGATGTTGGT
25 251 CTCACATTGG AGATCCCCAA GAGTGTC25ACT ATGAAGAATG TG25TAGTAACT
301 ACCACTCCTC CCTCAATTCA GAATGGAACA TACCGTTTCT GCTGTTGTAG
351 CACAGATTTA TGTAATGTCA ACTTTACTGA GAATTTTCCA CCTCCTGACA
401 CAACACCACT CAGTCCACCT CATTCATTTA ACCGAGATGA GACCGGTGGT
451 GGA40ACTCACA CATGCCACCT GTGCC40CAGCA CCTGA40ACTCC TGGGGGGACC
30 501 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
 5 851 GGAAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
 901 TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA
 951 GAACAACACTAC AAGACCACGC CTCCCGTGCT GAAGTCCGAC GGCTCCTTCT
 1001 TCCTCTATAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
 1051 GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA
 10 1101 GAAGAGCCTC TCCCTGTCTC CGGGTAAA (SEQ ID NO: 108)

The mature BMPRII-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 109) and may optionally be provided with the C-terminal lysine removed.

1 SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
 51 INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
 15 101 FTENFPPPD TPLSPPHSFN RDETTGGGTHT 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
 20 351 GK (SEQ ID NO: 109)

As described in Example 1, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 137) uses the TPA leader and incorporates an optional N-terminal extension. To promote formation of the BMPRII-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with anionic residues) can be introduced into the monomeric Fc polypeptide. The amino acid sequence of SEQ ID NO: 137 may optionally be provided with the C-terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic acid of SEQ ID NO: 138), and the mature monomeric Fc polypeptide (SEQ ID NO: 139) may optionally be provided with the C-terminal lysine removed.

The BMPRII-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 103 and SEQ ID NO: 139, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising BMPRII-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the BMPRII-Fc and Fc polypeptide sequences of SEQ ID NOs: 405-406 and 425-426,

5 respectively.

The BMPRII-Fc fusion polypeptide (SEQ ID NO: 405) uses the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
51  HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
10  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 KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN
15  351  VFSCSVMEHA LHNHYTQKSL SLSPGK          (SEQ ID NO: 405)

```

The leader sequence and linker are underlined. To promote formation of the BMPRII-Fc: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 tryptophan) can be introduced into the Fc domain of the BMPRII fusion polypeptide as indicated by

20 double underline above. The amino acid sequence of SEQ ID NO: 405 may optionally be provided with the C-terminal lysine removed.

The mature BMPRII-Fc fusion polypeptide (SEQ ID NO: 406) is as follows and may optionally be provided with the C-terminal lysine removed.

```

1  SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
25  51  INLVKQGCWS HIGDPQECHY EECVVTTPP SIQNGTYRFC CCSTDLCNVN
101  FTENFPPPD TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251  PQVYTLPPCR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTT
30  301  PVLDSGSGFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  GK          (SEQ ID NO: 406)

```

As described in Example 1, the complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 425) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the BMPRII-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, four amino acid substitutions can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 425 and the mature Fc polypeptide (SEQ ID NO: 426) may optionally be provided with the C-terminal lysine removed.

The BMPRII-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 406 and SEQ ID NO: 426, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising BMPRII-Fc:Fc.

Purification of various BMPRII-Fc: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.

A BiacoreTM-based binding assay was used to compare ligand binding selectivity of the single-arm BMPRII-Fc heterodimeric complex described above with that of an BMPRII-Fc homodimeric complex. The single-arm BMPRII-Fc and homodimeric BMPRII-Fc 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.

Ligand binding by single-arm BMPRII-Fc compared to BMPRII-Fc homodimer						
Ligand	BMPRII-Fc homodimer			Single-arm BMPRII-Fc		
	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 x 10 ⁷	7.5 x 10 ⁻²	3800	Minimal binding		
BMP2	Transient *		>2 x 10 ⁶	No binding		
BMP4	---			No binding		
BMP5	---			4.1 x 10 ⁵	1.5 x 10 ⁻²	36000
BMP6	Transient *		>8900	No binding		
BMP7	Transient *		>38000	No binding		

BMP9	1.2 x 10 ⁷	2.6 x 10 ⁻²	2100	Minimal binding		
BMP10	2.6 x 10 ⁷	2.5 x 10 ⁻³	98	2.1 x 10 ⁷	9.1 x 10 ⁻³	430
BMP15	9.9 x 10 ⁶	2.8 x 10 ⁻³	280	7.1 x 10 ⁷	6.7 x 10 ⁻²	940
GDF6	Transient *		>88000	Minimal binding		
GDF7	---			Transient *		>190000
* Indeterminate due to transient nature of interaction						
--- Not tested						

These comparative binding data indicate that single-arm BMPRII-Fc heterodimer retains binding to only a subset of ligands bound by BMPRII-Fc homodimer. In particular, while the single-arm BMPRII-Fc heterodimer retains binding to BMP10 and BMP15, binding to BMP9 is essentially eliminated.

Example 5. Generation of a single-arm MISRII-Fc heterodimer

Applicants envision construction of a soluble single-arm MISRII-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human MISRII is fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and MISRII-Fc fusion polypeptide, respectively, and the sequences for each are provided below. Applicants also envision additional single-arm MISRII-Fc heterodimeric complexes comprising the extracellular domain of MISRII isoform 2 or 3 (SEQ ID NOs: 76, 80).

Formation of a single-arm MISRII-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the MISRII-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 110-112 and 137-139, 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 MISRII-Fc fusion polypeptide employs the TPA leader and is as follows:

```

1  MDAMKRGGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
51 DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD

```

101 PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
 151 SIWMALTGGG THTCP^{PC}PAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
 201 VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
 251 WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ
 5 301 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLKSDG SFFLYSKLTV
 351 DKSRWQQGNV FSCSVMEAL HNHYTQKSLS LSPGK (SEQ ID NO: 110)

The leader and linker sequences are underlined. To promote formation of the MISRII-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (MISRII-Fc:MISRII-Fc or Fc:Fc), two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the Fc domain of the fusion polypeptide as indicated by
 10 double underline above. The amino acid sequence of SEQ ID NO: 110 may optionally be provided with the C-terminal lysine removed.

The mature MISRII-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 112) and may optionally be provided with the C-terminal lysine removed.

15 1 PPNRRTC^VFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51 TQDRAQVEMQ GCRDSDEPGC ESLHCDPSR AHPSPGSTLF TCSCGTDFCN
 101 ANYSHLPPPG SPGTPGSQGP QAAPGESIWM ALTGGGTHTC PPCPAPELLG
 151 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
 201 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
 20 251 SKAKGQPREP QVYTLPPSRK EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ
 301 PENNYKTTPP VLKSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
 351 TQKSLSLSPG K (SEQ ID NO: 112)

As described in Example 1, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 137) employs the TPA leader and incorporates an optional N-
 25 terminal extension. To promote formation of the MISRII-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with anionic residues) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 137 may optionally be provided with the C-terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic
 30 acid of SEQ ID NO: 138, and the mature monomeric Fc polypeptide (SEQ ID NO: 139) may optionally be provided with the C-terminal lysine removed.

The MISRII-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 112 and SEQ ID NO: 139, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising MISRII-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the MISRII-Fc and Fc polypeptide sequences of SEQ ID NOs: 407-408 and 425-426, respectively.

The MISRII-Fc fusion polypeptide (SEQ ID NO: 407) uses the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
51 DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101 PSPRAHPSPG STLFTCS CGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151 SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
15 201 VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251 WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ
301 VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPVLDSDG SFFLYSKLTV
351 DKSRWQQGNV FSCSVMEAL HNHYTQKSLS LSPGK
      (SEQ ID NO: 407)

```

The leader sequence and linker are underlined. To promote formation of the MISRII-Fc: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 tryptophan) can be introduced into the Fc domain of the MISRII fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 407 may optionally be provided with the C-terminal lysine removed.

The mature MISRII-Fc fusion polypeptide (SEQ ID NO: 408) is as follows and may optionally be provided with the C-terminal lysine removed.

```

1  PPNRRTC VFF EAPGVRG STK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
51 TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
30 101 ANYSHLPPPG SPGTPGSQGP QAAPGESIWM ALTGGGTHTC PPCPAPELLG
151 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
201 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI

```

251 SKAKGQPREP QVYTLPPCRE EMTKNQVSLW CLVKGFYPSD IAVEWESNGQ
 301 PENNYKTTPP VLDS DGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
 351 TQKSLSLSPG K (SEQ ID NO: 408)

As described in Example 1, the complementary form of monomeric G1Fc polypeptide
 5 (SEQ ID NO: 425) employs the TPA leader and incorporates an optional N-terminal
 extension. To promote formation of the MISRII-Fc:Fc heterodimer rather than either of the
 possible homodimeric complexes, four amino acid substitutions can be introduced into the
 monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 425 and
 the mature Fc polypeptide (SEQ ID NO: 426) may optionally be provided with the C-
 10 terminal lysine removed.

The MISRII-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO:
 408 and SEQ ID NO: 426, respectively, may be co-expressed and purified from a CHO cell
 line to give rise to a single-arm heteromeric protein complex comprising MISRII-Fc:Fc.

Purification of various MISRII-Fc:Fc complexes could be achieved by a series of
 15 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.

20 **Example 6. Generation and characterization of a single-arm TGFβRII-Fc heterodimer**

Applicants constructed a soluble single-arm TGFβRII-Fc heterodimeric complex
 comprising a monomeric Fc polypeptide with a short N-terminal extension and a second
 polypeptide in which the extracellular domain of human TGFβRII (short isoform, SEQ ID
 NO: 43) was fused to a separate Fc domain with a linker positioned between the extracellular
 25 domain and this second Fc domain. The individual constructs are referred to as monomeric
 Fc polypeptide and TGFβRII-Fc fusion polypeptide, respectively, and the sequences for each
 are provided below. Applicants also envision additional single-arm TGFβRII-Fc complexes
 comprising the extracellular domain of TGFβRII isoform A (SEQ ID NO: 68) as well as
 single-arm TGFβRII-Fc complexes in which the extracellular domain of canonical TGFβRII
 30 (short isoform, SEQ ID NO: 43) or that of TGFβRII isoform A (SEQ ID NO: 68) contain a
 36-amino-acid insert (SEQ ID NO: 95) derived from TGFβRII isoform C as described herein.

Formation of a single-arm TGF β RII-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the TGF β RII-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 113-115 and 137-139, 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 TGF β RII-Fc fusion polypeptide employs the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCAVAV RKNDENITLE
10  101  TVCHDPKLPY HDFILEDAAAS 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
15  351  SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

      (SEQ ID NO: 113)

```

The leader and linker sequences are underlined. To promote formation of the TGF β RII-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (TGF β RII-Fc: TGF β RII-Fc or Fc:Fc), two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the Fc domain of the fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 113 may optionally be provided with the C-terminal lysine removed.

This TGF β RII-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 114).

```

25      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
30  251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
      301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
      351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAAG CCTGGTGAGA

```

401 CTTTCTTCAT GTGTTCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451 TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAAGTCA
 501 CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT
 551 TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
 5 601 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
 10 851 GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGAAGGAG
 901 ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC
 951 CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACCT
 1001 ACAAGACCAC GCCTCCCGTG CTGAAGTCCG ACGGCTCCTT CTTCTCTAT
 1051 AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC
 15 1101 ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC
 1151 TCTCCCTGTC TCCGGGTAAA (SEQ ID NO: 114)

The mature TGF β RII-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 115) and may optionally be provided with the C-terminal lysine removed.

1 TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 20 51 ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
 101 MKEKKKPGET FFMCS CSSDE CNDNIIFSEE YNTSNPDTGG GTHTCPPCPA
 151 PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
 201 VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVS NKALPAP
 251 IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW
 25 301 ESNGQPENNY KTTTPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
 351 LHNHYTQKSL SLSPGK (SEQ ID NO: 115)

As described in Example 1, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 137) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the TGF β RII-Fc:Fc heterodimer rather than
 30 either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with anionic residues) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 137 may optionally be provided with the C-terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic

acid of SEQ ID NO: 138, and the mature monomeric Fc polypeptide (SEQ ID NO: 139) may optionally be provided with the C-terminal lysine removed.

The TGF β RII-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 115 and SEQ ID NO: 139, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising TGF β RII-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the TGF β RII-Fc and Fc polypeptide sequences of SEQ ID NOs: 409-410 and 425-426, respectively.

The TGF β RII-Fc fusion polypeptide (SEQ ID NO: 409) uses the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCAVAVW RKNDENITLE
15 101 TVCHDPKLPY HFILEDAAS 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
20 351 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
      (SEQ ID NO: 409)

```

The leader sequence and linker are underlined. To promote formation of the TGF β RII-Fc: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 tryptophan) can be introduced into the Fc domain of the TGF β RII fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 409 may optionally be provided with the C-terminal lysine removed.

The mature TGF β RII-Fc fusion polypeptide (SEQ ID NO: 410) is as follows and may optionally be provided with the C-terminal lysine removed.

```

30 1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
51 ITSICEKPQE VCAVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101 MKEKKKPGET FFMCS CSSDE CNDNIIFSEE YNTSNPDTGG GTHTCPPCPA

```

151 PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
 201 VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVS NKALPAP
 251 IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW
 301 ESNQGPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
 5 351 LHNHYTQKSL SLSPGK (SEQ ID NO: 410)

As described in Example 1, the complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 425) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the TGFβRII-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, four amino acid substitutions can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 425 and the mature monomeric Fc polypeptide (SEQ ID NO: 426) may optionally be provided with the C-terminal lysine removed.

The TGFβRII-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 410 and SEQ ID NO: 426, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising TGFβRII-Fc:Fc.

Purification of various TGFβRII-Fc: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.

A Biacore™-based binding assay was used to compare ligand binding selectivity of the single-arm TGFβRII-Fc heterodimeric complex described above with that of an TGFβRII-Fc homodimeric complex. The single-arm TGFβRII-Fc and homodimeric TGFβRII-Fc 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.

Ligand binding by single-arm TGFβRII-Fc compared to TGFβRII-Fc homodimer						
Ligand	TGFβRII-Fc homodimer			Single-arm TGFβRII-Fc		
	k _a (1/Ms)	k _d (1/s)	K _D (pM)	k _a (1/Ms)	k _d (1/s)	K _D (pM)
TGFβ1	4.2 x 10 ⁷	1.1 x 10 ⁻³	25	1.5 x 10 ⁸	4.7 x 10 ⁻³	31

TGFβ2	Transient*		> 44000	Transient*		> 61000
TGFβ3	5.9 x 10 ⁷	5.9 x 10 ⁻³	99	1.4 x 10 ⁸	9.9 x 10 ⁻³	73
* Indeterminate due to transient nature of interaction						

Example 7. Generation and characterization of a single-arm ALK1-Fc heterodimer

Applicants constructed a soluble single-arm ALK1-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human ALK1 was fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and ALK1-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

Formation of a single-arm ALK1-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the ALK1-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 116-118 and 140-142, 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 ALK1-Fc fusion polypeptide employs the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
51  GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
101 NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
20  151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251  PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP
301  PVLDSDGSEFF LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  G (SEQ ID NO: 116)

```

The leader and linker sequences are underlined. To promote formation of the ALK1-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ALK1-Fc:ALK1-Fc or Fc:Fc), two amino acid substitutions (replacing lysines with anionic amino acids) can be introduced into the Fc domain of the fusion polypeptide as indicated by double

underline above. The amino acid sequence of SEQ ID NO: 116 may optionally be provided with a lysine added at the C-terminus.

This ALK1-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 117).

```

5      1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
      51  AGTCTTCGTT TCGCCCGGCG CCGACCCTGT GAAGCCGTCT CGGGGCCCCG
101    TGGTGACCTG CACGTGTGAG AGCCCACATT GCAAGGGGCC TACCTGCCGG
      151  GGGGCCTGGT GCACAGTAGT GCTGGTGCGG GAGGAGGGGA GGCACCCCCA
      201  GGAACATCGG GGCTGCGGGA ACTTGACACAG GGAGCTCTGC AGGGGCCGCC
10      251  CCACCGAGTT CGTCAACCAC TACTGCTGCG ACAGCCACCT CTGCAACCAC
      301  AACGTGTCCC TGGTGCTGGA GGCCACCCAA CCTCCTTCGG AGCAGCCGGG
      351  AACAGATGGC CAGCTGGCCA CCGGTGGTGG AACTCACACA TGCCCACCGT
      401  GCCCAGCACC TGAACTCCTG GGGGACCGT CAGTCTTCCT CTTCCCCCCA
      451  AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT
15      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
20      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
25     1001  ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
      1051  GGT          (SEQ ID NO: 117)

```

The mature ALK1-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 118) and may optionally be provided with a lysine added at the C-terminus.

```

      1  DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRCGN
30      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: 118)

As described in Example 2, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 140) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK1-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 140 may optionally be provided with the C-terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic acid of SEQ ID NO: 141, and the mature monomeric Fc polypeptide (SEQ ID NO: 142) may optionally be provided with the C-terminal lysine removed.

The ALK1-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 118 and SEQ ID NO: 142, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK1-Fc:Fc.

In another approach to promoting 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 ALK1-Fc and Fc polypeptide sequences of SEQ ID NOs: 411-412 and 427-428, respectively.

The ALK1-Fc fusion polypeptide (SEQ ID NO: 411) uses the TPA leader and is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGADPKPS 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: 411)

The leader sequence and linker are underlined. To promote formation of the ALK1-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, 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: 411 may optionally be provided with the C-terminal lysine removed.

The mature ALK1-Fc fusion polypeptide (SEQ ID NO: 412) is as follows and may optionally be provided with the C-terminal lysine removed.

```

5      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
10    251  KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLVSK LTVDKSRWQQ
     301  GNVFSCSVMH EALHNNHYTQK SLSLSPGK          (SEQ ID NO: 412)

```

As described in Example 2, the complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 427) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK1-Fc: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 tryptophan) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 427 and the mature Fc polypeptide (SEQ ID NO: 428) may optionally be provided with the C-terminal lysine removed.

The ALK1-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 412 and SEQ ID NO: 428, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK1-Fc:Fc.

Purification of various ALK1-Fc: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.

A BiacoreTM-based binding assay was used to compare ligand binding selectivity of the single-arm ALK1-Fc heterodimeric complex described above with that of an ALK1-Fc homodimeric complex. The single-arm ALK1-Fc and homodimeric ALK1-Fc 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) typically associated with the most effective ligand traps are denoted by gray shading.

Ligand binding by single-arm ALK1-Fc compared to ALK1-Fc homodimer						
Ligand	ALK1-Fc homodimer			Single-arm ALK1-Fc		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
BMP9	7.9×10^6	1.3×10^{-4}	16	1.2×10^7	1.7×10^{-3}	140
BMP10	1.7×10^7	1.1×10^{-4}	6	2.8×10^6	3.0×10^{-4}	100

These comparative binding data indicate that single-arm ALK1-Fc has altered ligand selectivity compared to homodimeric ALK1-Fc. Single-arm ALK1-Fc retains the strong binding to BMP10 observed with homodimeric ALK1-Fc while binding BMP9 less tightly than does ALK1-Fc homodimer, as the off-rate of BMP9 binding to single-arm ALK1-Fc is approximately 10-fold faster than it is for binding to homodimeric ALK1-Fc. These results indicate that single-arm ALK1-Fc is a more selective antagonist than ActRIIB-Fc homodimer. Accordingly, single-arm ALK1-Fc will be more useful than homodimeric ALK1-Fc in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of BMP10 but reduce antagonism of BMP9.

Example 8. Generation of a single-arm ALK2-Fc heterodimer

Applicants envision construction of a soluble single-arm ALK2-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human ALK2 is fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and ALK2-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

Formation of a single-arm ALK2-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the ALK2-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 119-121 and 140-142, 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 ALK2-Fc fusion polypeptide employs the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSGCNED
5  51  HCEGQQCFSS LSINDGFHVV QKGCFFQVYEQ GKMTCKTPPS PGQAVECCQG
101 DWCNRNITAQ LPTKGKSFPQ TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201 REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG
251 QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
10 301 DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351 SLSPG (SEQ ID NO: 119)

```

The leader and linker sequences are underlined. To promote formation of the ALK2-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ALK2-Fc:ALK2-Fc or Fc:Fc), two amino acid substitutions (replacing lysines with anionic amino acids) can be introduced into the Fc domain of the fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 119 may optionally be provided with a lysine added at the C-terminus.

This ALK2-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 120).

```

20  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51  AGTCTTCGTT TCGCCCGGCG CCATGGAAGA TGAGAAGCCC AAGGTCAACC
101 CCAAACCTCTA CATGTGTGTG TGTGAAGGTC TCTCCTGCGG TAATGAGGAC
151 CACTGTGAAG GCCAGCAGTG CTTTTCCTCA CTGAGCATCA ACGATGGCTT
201 CCACGTCTAC CAGAAAGGCT GCTTCCAGGT TTATGAGCAG GGAAAGATGA
25 251 CCTGTAAGAC CCCGCCGTCC CCTGGCCAAG CTGTGGAGTG CTGCCAAGGG
301 GACTGGTGTA ACAGGAACAT CACGGCCCAG CTGCCCACTA AAGGAAAATC
351 CTTCCCTGGA ACACAGAATT TCCACTTGGA GACCGGTGGT GGAATCACA
401 CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
451 CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
30 501 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 CCCCATCCC GGGAGGAGAT
 801 GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA
 5 851 GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACACTAC
 901 GACACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTATAG
 951 CGACCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
 1001 GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
 1051 TCCCTGTCTC CGGGT (SEQ ID NO: 120)

10 The mature ALK2-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 121)
 and may optionally be provided with a lysine added at the C-terminus.

1 MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVVYQKGC
 51 FQVYEQ GKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPQTQNF
 101 HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 15 151 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 201 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
 251 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDS DGSFF LYSDLTVDKS
 301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G (SEQ ID NO: 121)

As described in Example 2, the complementary form of monomeric human G1Fc
 20 polypeptide (SEQ ID NO: 140) employs the TPA leader and incorporates an optional N-
 terminal extension. To promote formation of the ALK2-Fc:Fc heterodimer rather than either
 of the possible homodimeric complexes, two amino acid substitutions (replacing anionic
 residues with lysines) can be introduced into the monomeric Fc polypeptide as indicated.
 The amino acid sequence of SEQ ID NO: 140 may optionally be provided with the C-
 25 terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic acid
 of SEQ ID NO: 141, and the mature monomeric Fc polypeptide (SEQ ID NO: 142) may
 optionally be provided with the C-terminal lysine removed.

The ALK2-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 121
 and SEQ ID NO: 142, respectively, may be co-expressed and purified from a CHO cell line
 30 to give rise to a single-arm heteromeric protein complex comprising ALK2-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using
 asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary

hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ALK2-Fc and Fc polypeptide sequences of SEQ ID NOs: 413-414 and 427-428, respectively.

The ALK2-Fc fusion polypeptide (SEQ ID NO: 413) uses the TPA leader and is as follows:

```

5      1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSGCGNED
      51  HCEGQQCFSS LSINDGFHVY QKGC FQVYEQ GKMTCKTPPS PGQAVECCQG
     101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
     151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFENWYVDG VEVHNAKTKP
     201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG
    10  251  QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY
     301  KTTTPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
     351  SLSPGK          (SEQ ID NO: 413)
  
```

The leader sequence and linker are underlined. To promote formation of the ALK2-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, four amino acid substitutions can be introduced into the Fc domain of the ALK2 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 413 may optionally be provided with the C-terminal lysine removed.

The mature ALK2-Fc fusion polypeptide (SEQ ID NO: 414) is as follows and may optionally be provided with the C-terminal lysine removed.

```

20      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
    25  251  SCAVKGFYPS DIAVEWESNG QPENNYKTP PVLDSDGSFF LVSKLTVDKS
     301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK          (SEQ ID NO: 414)
  
```

As described in Example 2, the complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 427) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK2-Fc: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 tryptophan) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 427 and the mature Fc

polypeptide (SEQ ID NO: 428) may optionally be provided with the C-terminal lysine removed.

The ALK2-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 414 and SEQ ID NO: 428, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK2-Fc:Fc.

Purification of various ALK2-Fc: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 9. Generation of a single-arm ALK4-Fc heterodimer

Applicants envision construction of a soluble single-arm ALK4-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human ALK4 is fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and ALK4-Fc fusion polypeptide, respectively, and the sequences for each are provided below. Applicants also envision additional single-arm ALK4-Fc heterodimeric complexes comprising the extracellular domain of ALK4 isoform B (SEQ ID NO: 84).

Formation of a single-arm ALK4-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the ALK4-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 125-127 and 140-142, 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 ALK4-Fc fusion polypeptide employs the TPA leader and is as follows:

```

1  MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
51  GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSD LRNTHCCYTD
30 101 YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP

```

201 REEQYNSTYR VSVLTVLHQ DWLNGKEYKC KVS NKALPAP IEKTISKAKG
 251 QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
 301 DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
 351 SLSPG (SEQ ID NO: 125)

5 The leader and linker sequences are underlined. To promote formation of the ALK4-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ALK4-Fc:ALK4-Fc or Fc:Fc), two amino acid substitutions (replacing lysines with anionic amino acids) can be introduced into the Fc domain of the fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 125 may optionally be provided
 10 with a lysine added at the C-terminus.

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 126).

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51 AGTCTTCGTT TCGCCCGGCG CCTCCGGGCC CCGGGGGGTC CAGGCTCTGC
 15 101 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
 20 351 TGAGCACCCG TCCATGTGGG GCCCGGTGGA GACCGGTGGT GGAAC TCACA
 401 CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
 451 CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
 501 GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
 551 TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
 25 601 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
 30 851 GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACA ACTAC
 901 GACACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTATAG
 951 CGACCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
 1001 GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC

1051 TCCCTGTCTC CGGGT (SEQ ID NO: 126)

The mature ALK4-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 127) and may optionally be provided with a lysine added at the C-terminus.

1 SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
 5 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 PVLDSGGSFF LYSDLTVDKS
 10 301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G (SEQ ID NO: 127)

As described in Example 2, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 140) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK4-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 140 may optionally be provided with the C-terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic acid of SEQ ID NO: 141, and the mature monomeric Fc polypeptide (SEQ ID NO: 142) may optionally be provided with the C-terminal lysine removed.

20 The ALK4-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 127 and SEQ ID NO: 142, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK4-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ALK4-Fc and Fc polypeptide sequences of SEQ ID NOs: 417-418 and 427-428, respectively.

The ALK4-Fc fusion polypeptide (SEQ ID NO: 417) uses the TPA leader and is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 30 51 GACMVSI FNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
 101 YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
 151 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP

201 REEQYNSTYR VSVLTVLHQ DWLNGKEYKC KVS NKALPAP IEKTISKAKG
 251 QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY
 301 KTTTPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
 351 SLSPGK (SEQ ID NO: 417)

5 The leader sequence and linker are underlined. To promote formation of the ALK4-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, 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: 417 may optionally be provided with the C-terminal lysine removed.

10 The mature ALK4-Fc fusion polypeptide (SEQ ID NO: 418) is as follows and may optionally be provided with the C-terminal lysine removed.

1 SGPRGVQALL CACTSCLQAN YTCETDGACM VSI FNLDGME HHVRTCIPKV
 51 ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
 101 PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 15 151 VSHEDPEVKF NWFVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 201 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
 251 SCAVKGFYPS DIAVEWESNG QPENNYKTTT PVLDSDGSFF LVSKLTVDKS
 301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 418)

As described in Example 2, the complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 427) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK4-Fc: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 tryptophan) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 427 and the mature Fc polypeptide (SEQ ID NO: 428) may optionally be provided with the C-terminal lysine removed.

The ALK4-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 418 and SEQ ID NO: 428, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK4-Fc:Fc.

30 Purification of various ALK4-Fc: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. Generation of a single-arm ALK5-Fc heterodimer

Applicants envision construction of a soluble single-arm ALK5-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the extracellular domain of human ALK5 is fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and ALK5-Fc fusion polypeptide, respectively, and the sequences for each are provided below. Applicants also envision additional single-arm ALK5-Fc heterodimeric complexes comprising the extracellular domain of ALK5 isoform 2 (SEQ ID NO: 88).

Formation of a single-arm ALK5-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the ALK5-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 128-130 and 140-142, 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 ALK5-Fc fusion polypeptide employs the TPA leader and 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
25 251 EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYDTT
301 PPVLDSGGSF FLYSDLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
351 PG (SEQ ID NO: 128)

```

The leader and linker sequences are underlined. To promote formation of the ALK5-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ALK5-Fc:ALK5-Fc or Fc:Fc), two amino acid substitutions (replacing lysines with anionic amino acids) can be introduced into the Fc domain of the fusion polypeptide as indicated. The

amino acid sequence of SEQ ID NO: 128 may optionally be provided with a lysine added at the C-terminus.

This ALK5-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 129).

```

5      1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
      51  AGTCTTCGTT TCGCCCGGCG CCGCGCTGCT CCCGGGGGCG ACGGCGTTAC
     101  AGTGTTTCTG CCACCTCTGT ACAAAGACA ATTTTACTTG TGTGACAGAT
     151  GGGCTCTGCT TTGTCTCTGT CACAGAGACC ACAGACAAAG TTATACACAA
     201  CAGCATGTGT ATAGCTGAAA TTGACTTAAT TCCTCGAGAT AGGCCGTTTG
    10  251  TATGTGCACC CTCTTCAAAA ACTGGGTCTG TGACTIONAAC ATATTGCTGC
     301  AATCAGGACC ATTGCAATAA AATAGAACTT CCAACTACTG TAAAGTCATC
     351  ACCTGGCCTT GGTCTGTGG AAACCGGTGG TGGAATCAC ACATGCCAC
     401  CGTGCCAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC
     451  CCAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG
    15  501  CGTGGTGGTG GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT
     551  ACGTGGACGG CGTGGAGGTG CATAATGCCA AGACAAAGCC GCGGGAGGAG
     601  CAGTACAACA GCACGTACCG TGTGGTCAGC GTCCTCACCG TCCTGCACCA
     651  GGACTIONCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC AACAAAGCCC
     701  TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
    20  751  GAACCACAGG TGTACACCCT GCCCCATCC CGGGAGGAGA TGACCAAGAA
     801  CCAGGTCAGC CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG
     851  CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG AGAACAACTA CGACACCACG
     901  CCTCCCGTGC TGGACTCCGA CGGCTCCTT TTCCTCTATA GCGACCTCAC
     951  CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA
    25 1001  TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
     1051  CCGGGT (SEQ ID NO: 129)

```

The mature ALK5-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 130) and may optionally be provided with a lysine added at the C-terminus.

```

      1  ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
    30  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: 130)

As described in Example 2, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 140) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK5-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 140 may optionally be provided with the C-terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic acid of SEQ ID NO: 141, and the mature monomeric Fc polypeptide (SEQ ID NO: 142) may optionally be provided with the C-terminal lysine removed.

The ALK5-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 130 and SEQ ID NO: 142, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK5-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ALK5-Fc and Fc polypeptide sequences of SEQ ID NOs: 419-420 and 427-428, respectively.

The ALK5-Fc fusion polypeptide (SEQ ID NO: 419) uses the TPA leader and is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
 51 GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
 101 NQDHCNKIEL PTTVKSSPGL GPVETTGGGTH TCPPCPAPEL LGGPSVFLFP
 151 PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
 201 QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 251 EPQVCTLPPS REEMTKNQVS LSCAVKGFYP SDIAVEWESN GQPENNYKTT
 301 PPVLDS DGSF FLVSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
 351 PGK (SEQ ID NO: 419)

The leader sequence and linker are underlined. To promote formation of the ALK5-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, four amino acid substitutions can be introduced into the Fc domain of the ALK5 fusion polypeptide as

indicated by double underline above. The amino acid sequence of SEQ ID NO: 419 may optionally be provided with the C-terminal lysine removed.

The mature ALK5-Fc fusion polypeptide (SEQ ID NO: 420) is as follows and may optionally be provided with the C-terminal lysine removed.

```

5      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
10    251  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLVS KLTVDKSRWQ
     301  QGNVFSCSVM HEALHNHYTQ KSLSLSPGK      (SEQ ID NO: 420)

```

As described in Example 2, the complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 427) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK5-Fc: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 tryptophan) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 427 and the mature monomeric G1Fc polypeptide (SEQ ID NO: 428) may optionally be provided with the C-terminal lysine removed.

The ALK5-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 420 and SEQ ID NO: 428, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK5-Fc:Fc.

Purification of various ALK5-Fc: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 11. Generation of a single-arm ALK6-Fc heterodimer

Applicants envision construction of a soluble single-arm ALK6-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a

second polypeptide in which the extracellular domain of human ALK6 is fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and ALK6-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

- 5 Applicants also envision additional single-arm ALK6-Fc heterodimeric complexes comprising the extracellular domain of ALK6 isoform 2 (SEQ ID NO: 92).

Formation of a single-arm ALK6-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the ALK6-Fc and monomeric Fc polypeptide sequences of SEQ ID
10 NOs: 131-133 and 140-142, 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 ALK6-Fc fusion polypeptide employs the TPA leader and is as follows:

```

1   MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
15  51  EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLEGSDF QCRDTPIPHQ
    101  RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
    151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFENWYVDG
    201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP
    251  IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW
20  301  ESNQQPENNY DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA
    351  LHNHYTQKSL SLSPG          (SEQ ID NO: 131)

```

The leader and linker sequences are underlined. To promote formation of the ALK6-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ALK6-Fc:ALK6-Fc or Fc:Fc), two amino acid substitutions (replacing lysines with anionic amino
25 acids) can be introduced into the Fc domain of the fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 131 may optionally be provided with a lysine added at the C-terminus.

This ALK6-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 132).

```

30  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 GGTCACCTTCT GGTTGCCTAG
 251 GACTAGAAGG CTCAGATTTT CAGTGTCTGGG ACACTCCCAT TCCTCATCAA
 301 AGAAGATCAA TTGAATGCTG CACAGAAAGG AACGAATGTA ATAAAGACCT
 5 351 ACACCCTACA CTGCCTCCAT TGAAAAACAG AGATTTTGTGTT GATGGACCTA
 401 TACACCACAG GACCGGTGGT GGAAGTCACA CATGCCCACC GTGCCCAGCA
 451 CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA
 501 GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG
 551 ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC
 10 601 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
 15 851 TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
 901 GAGAGCAATG GGCAGCCGGA GAACAACTAC GACACCACGC CTCCCGTGCT
 951 GGAATCCGAC GGCTCCTTCT TCCTCTATAG CGACCTCACC GTGGACAAGA
 1001 GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT
 1051 CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGT
 20 (SEQ ID NO: 132)

The mature ALK6-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 133) 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
 25 101 KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
 151 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
 201 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
 251 EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTP PVLDSDGSFF
 301 LYS~~D~~LTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
 30 (SEQ ID NO: 133)

As described in Example 2, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 140) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK6-Fc:Fc heterodimer rather than either

of the possible homodimeric complexes, two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 140 may optionally be provided with the C-terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic acid of SEQ ID NO: 141, and the mature monomeric Fc protein (SEQ ID NO: 142) may optionally be provided with the C-terminal lysine removed.

The ALK6-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 133 and SEQ ID NO: 142, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK6-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ALK6-Fc and Fc polypeptide sequences of SEQ ID NOs: 421-422 and 427-428, respectively.

The ALK6-Fc fusion polypeptide (SEQ ID NO: 421) uses the TPA leader and is as follows:

```

1  MDAMKRG LCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
51 EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLEGSDF QCRDTPIPHQ
101 RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151 PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFENWYVDG
201 VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP
251 IEKTISKAKG QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW
301 ESNQGPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351 LHNHYTQKSL SLSPGK (SEQ ID NO: 421)

```

The leader sequence and linker are underlined. To promote formation of the ALK6-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, four amino acid substitutions can be introduced into the Fc domain of the ALK6 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 421 may optionally be provided with the C-terminal lysine removed.

The mature ALK6-Fc fusion polypeptide (SEQ ID NO: 422) is as follows and may optionally be provided with the C-terminal lysine removed.

```

1  KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
51 LPVVTSGCLG LEGSDFQCRD TPIPHQRRI ECCTERNECN KDLHPTLPPL

```

101 KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
 151 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
 201 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPSPR
 251 EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSGGSFF
 5 301 LVSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK

(SEQ ID NO: 422)

As described in Example 2, the complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 427) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK6-Fc: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 tryptophan) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 427 and the mature monomeric G1Fc polypeptide (SEQ ID NO: 428) may optionally be provided with the C-terminal lysine removed.

The ALK6-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 422 and SEQ ID NO: 428, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK6-Fc:Fc.

Purification of various ALK6-Fc: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. Generation of a single-arm ALK7-Fc heterodimer

Applicants envision construction of a soluble single-arm ALK7-Fc heterodimeric complex comprising a monomeric Fc polypeptide with a short N-terminal extension and a second polypeptide in which the N-terminally truncated (NΔ4) extracellular domain of human ALK7 is fused to a separate Fc domain with a linker positioned between the extracellular domain and this second Fc domain. The individual constructs are referred to as monomeric Fc polypeptide and ALK7-Fc fusion polypeptide, respectively, and the sequences for each are provided below. Applicants also envision additional single-arm ALK7-Fc heterodimeric complexes comprising other N-terminally truncated variants (e.g., NΔ5 variant)

of ALK7 isoform 1 (SEQ ID NO: 313), the extracellular domain of ALK7 isoform 2 (SEQ ID NO: 302), or native processed sequences of ALK7 isoforms 3 and 4 (SEQ ID NOs: 306, 310).

Formation of a single-arm ALK7-Fc heterodimer may be guided by approaches similar to those described for single-arm ActRIIB-Fc heterodimer in Example 1. In a first approach, illustrated in the ALK7-Fc and monomeric Fc polypeptide sequences of SEQ ID NOs: 134-136 and 140-142, 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 ALK7-Fc fusion polypeptide employs the TPA leader and is as follows:

```

10      1  MDAMKRGLCC VLLLCGAVFV SPGAGLKVC 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
15     251  EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSGGSFF
     301  LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
      (SEQ ID NO: 134)

```

The leader and linker sequences are underlined. To promote formation of the ALK7-Fc:Fc heterodimer rather than either of the possible homodimeric complexes (ALK7-Fc:ALK7-Fc or Fc:Fc), two amino acid substitutions (replacing lysines with anionic amino acids) can be introduced into the Fc domain of the fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 134 may optionally be provided with a lysine added at the C-terminus.

This ALK7-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 135).

```

25      1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
      51  AGTCTTCGTT TCGCCCGGCG CCGGACTGAA GTGTGTATGT CTTTGTGTG
     101  ATTCTTCAAA CTTTACCTGC CAAACAGAAG GAGCATGTTG GGCATCAGTC
     151  ATGCTAACCA ATGGAAGA GCAGGTGATC AAATCCTGTG TCTCCCTTCC
30     201  AGAACTGAAT GCTCAAGTCT TCTGTCATAG TTCCAACAAT GTTACCAAAA
     251  CCGAATGCTG CTTACAGAT TTTTGCAACA ACATAAACT GCACCTTCCA
     301  ACAGCATCAC CAAATGCCCC AAAACTTGA CCCATGGAGA CCGGTGGTGG

```

351 AACTCACACA TGCCACCCGT GCCCAGCACC TGAACCTCTG GGGGGACCGT
 401 CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG
 451 ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA
 501 GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA
 5 551 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
 10 801 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: 135)

15 The mature ALK7-Fc fusion polypeptide sequence is expected to be as follows (SEQ ID NO: 136) and may optionally be provided with a lysine added at the C-terminus.

1 GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51 CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGP MET GGGTHTCPPC
 101 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
 20 151 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
 201 APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
 251 EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ GNVFSCSVMH
 301 EALHNHYTQK SLSLSPG (SEQ ID NO: 136)

25 As described in Example 2, the complementary form of monomeric human G1Fc polypeptide (SEQ ID NO: 140) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK7-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing anionic residues with lysines) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 140 may optionally be provided with the C-
 30 terminal lysine removed. This complementary Fc polypeptide is encoded by the nucleic acid of SEQ ID NO: 141, and the mature monomeric Fc polypeptide (SEQ ID NO: 142) may optionally be provided with the C-terminal lysine removed.

The ALK7-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 136 and SEQ ID NO: 142, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK7-Fc:Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion polypeptides, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ALK7-Fc and Fc polypeptide sequences of SEQ ID NOs: 423-424 and 427-428, respectively.

The ALK7-Fc fusion polypeptide (SEQ ID NO: 423) uses the TPA leader and is as follows:

```

10      1  MDAMKRGLCC VLLLCGAVFV SPGAGLKVC 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
15     251  EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSGGSFF
     301  LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
      (SEQ ID NO: 423)

```

The leader sequence and linker are underlined. To promote formation of the ALK7-Fc:Fc heterodimer rather than either of the possible homodimeric complexes, four amino acid substitutions can be introduced into the Fc domain of the ALK7 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 423 may optionally be provided with the C-terminal lysine removed.

The mature ALK7-Fc fusion polypeptide (SEQ ID NO: 424) is expected to be as follows and may optionally be provided with the C-terminal lysine removed.

```

25      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
30     251  EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH
     301  EALHNHYTQK SLSLSPGK (SEQ ID NO: 424)

```

As described in Example 2, the complementary form of monomeric G1Fc polypeptide (SEQ ID NO: 427) employs the TPA leader and incorporates an optional N-terminal extension. To promote formation of the ALK7-Fc: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 tryptophan) can be introduced into the monomeric Fc polypeptide as indicated. The amino acid sequence of SEQ ID NO: 427 and the mature monomeric G1Fc polypeptide (SEQ ID NO: 428) may optionally be provided with the C-terminal lysine removed.

The ALK7-Fc fusion polypeptide and monomeric Fc polypeptide of SEQ ID NO: 424 and SEQ ID NO: 428, respectively, may be co-expressed and purified from a CHO cell line to give rise to a single-arm heteromeric protein complex comprising ALK7-Fc:Fc.

Purification of various ALK7-Fc: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.

Together these examples demonstrate that type I or type II receptor polypeptides, when placed in the context of a single-arm heteromeric protein complex, form novel binding pockets that exhibit altered selectivity relative to a homodimeric complex of the same receptor polypeptide, allowing the formation of novel protein agents for possible use as therapeutic agents.

We claim:

1. A soluble protein complex comprising a first polypeptide covalently or non-covalently associated with a second polypeptide, wherein:
 - a. the first polypeptide comprises an amino acid sequence of a first member of an interaction pair and an amino acid sequence of an ActRIIB polypeptide, which is at least 85% identical to amino acids 29-109 of SEQ ID NO: 1; and wherein the ActRIIB polypeptide comprises a leucine at the amino acid position corresponding to position 79 of SEQ ID NO: 1; and
 - b. the second polypeptide comprises an amino acid sequence of a second member of the interaction pair, and wherein the second polypeptide does not comprise a TGF β superfamily type I or type II receptor polypeptide; wherein the first member of the interaction pair comprises an Fc domain, and wherein the second member of the interaction pair comprises an Fc domain.

2. The protein complex of claim 1, wherein the ActRIIB polypeptide comprises an amino acid sequence that is:
 - a. at least 90%, 95%, 97%, 98%, 99% or 100% identical to the sequence of any of SEQ ID Nos: 1, 2, 3, 4, 5, and 6; or
 - b. at least 90%, 95%, 97%, 98%, 99% or 100% identical to a polypeptide that begins at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 of SEQ ID NO: 1, and ends 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.

3. The protein complex of claim 1 or claim 2, wherein the protein complex is a recombinant heterodimer.

4. The protein complex of any one of claims 1-3, wherein the first member of an interaction pair comprises a first constant region from an IgG heavy chain, and/or wherein the second member of an interaction pair comprises a second constant region from an IgG heavy chain.

5. The protein complex of claim 4, wherein the first constant region from an IgG heavy chain is a first immunoglobulin Fc domain, and/or wherein the second constant region from an IgG heavy chain is a first immunoglobulin Fc domain.
6. The protein complex of any one of claims 1-5, wherein the first polypeptide and/or second polypeptide comprises 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.
7. The protein complex of any one of claims 1-6, wherein the protein complex has one or more of the following characteristics: i) binds to a TGF-beta superfamily ligand with a K_D of less than or equal to 10^{-7} , 10^{-8} , 10^{-9} , or 10^{-10} M; and ii) inhibits a TGF-beta superfamily type I and/or type II receptor-mediated signaling transduction a cell.
8. The protein complex of any one of claims 1-7, wherein the protein complex binds to one or more of: BMP10, GDF8, GDF11/BMP11, activin A, or activin B.
9. The protein complex of any one of claims 1-8, wherein the protein complex inhibits the activity of one or more TGF-beta superfamily ligands in a cell-based assay; wherein the TGF-beta superfamily ligand is selected from: BMP10, GDF8, GDF11/BMP11, activin A, or activin B.
10. The protein complex of any one of claims 1-9, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 90% or 95% identical to amino acids 29-109 of SEQ ID NO: 1.
11. The protein complex of any one of claims 1-10, wherein the ActRIIB polypeptide comprises amino acids 29-109 of SEQ ID NO: 1.

12. The protein complex of any one of claims 1-11, wherein the first polypeptide comprises an amino acid sequence that is at least 90% or 95% identical to SEQ ID NO: 404, and wherein the second polypeptide comprises an amino acid sequence that is at least 90% or 95% identical to SEQ ID NO: 426.
13. The protein complex of any one of claims 1-11, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 404, and wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO: 426.
14. The protein complex of claim 5, wherein the immunoglobulin Fc domain is an Fc immunoglobulin domain of IgG1.
15. A pharmaceutical preparation comprising the protein complex of any one of claims 1-14 and a pharmaceutically acceptable carrier.
16. A method for treating a patient having a TGF β superfamily-associated condition, comprising administering to the patient the protein complex of any one of claims 1-14 or the pharmaceutical preparation of claim 15.
17. The method of claim 16, wherein the TGF β superfamily-associated condition is selected from the group: a muscle disorder, a red blood cell disorder, an anemia, a bone disorder, bone loss, a fibrotic disorder, chronic kidney disease, a metabolic disease, type II diabetes, obesity, and a cardiovascular disorder.
18. Use of a protein complex of any one of claims 1-14 or a pharmaceutical preparation of claim 15 for the manufacture of a medicament for the treatment of a TGF β superfamily-associated condition in a patient.

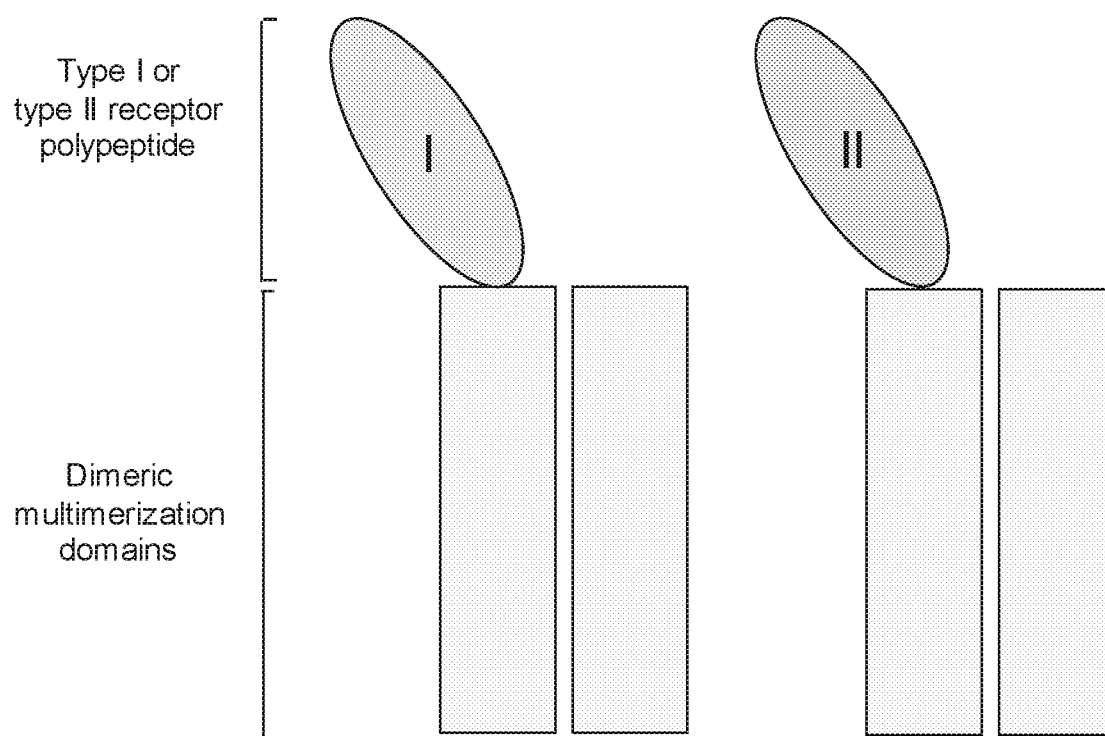


FIGURE 1

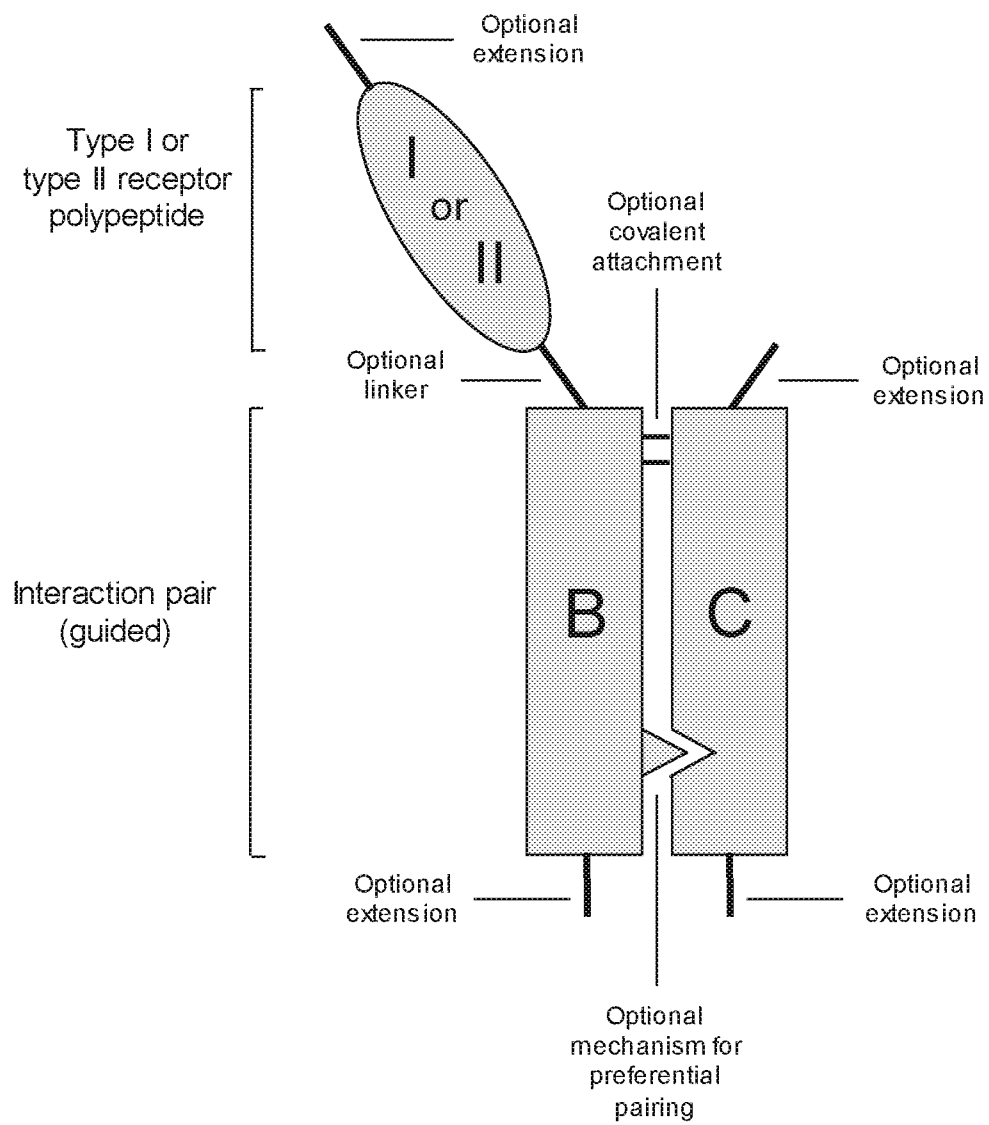


FIGURE 2

ActRIIa ILGRSETQEC IFFNANWIKD RTNQTGVEPC YSDKDEFRRHC FRTWENISGS
ActRIIb GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT

IEIVRQGCWL DDINCYLRFD CVERKDSPEV YRCCCEGNMC NERFSYFPFM
IELVKEGCWL DDFNCYLRQE CVATEENPQV YRCCCEGNFC NERFTHLPEA

EVTQPTSNPV TRKPP
GGPEVTYEPP PTAPT

FIGURE 3

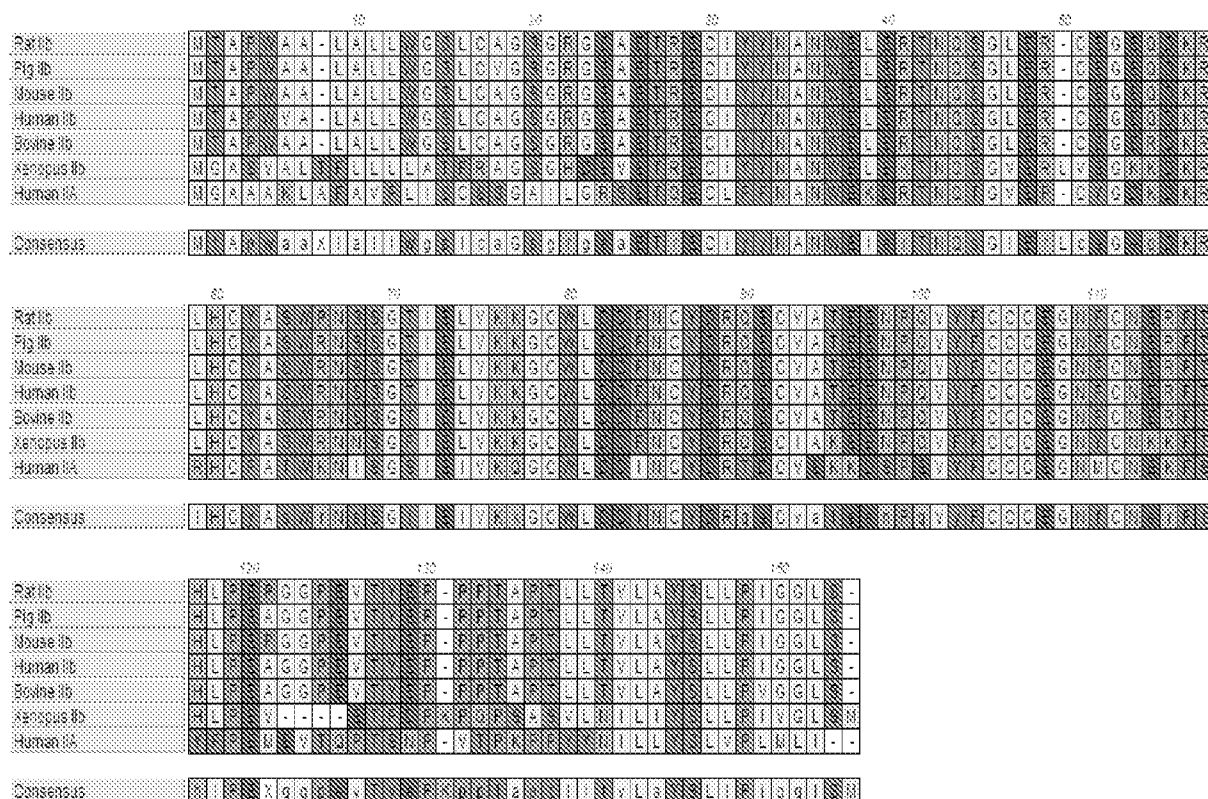


FIGURE 4

IgG1 PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK 225

IgG4 PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK 229

IgG2 FMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK 223

IgG3 FMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMEALHNRFTQKSLSLSPGK 232

*,*****;*****;*:*****;***** **

FIGURE 5

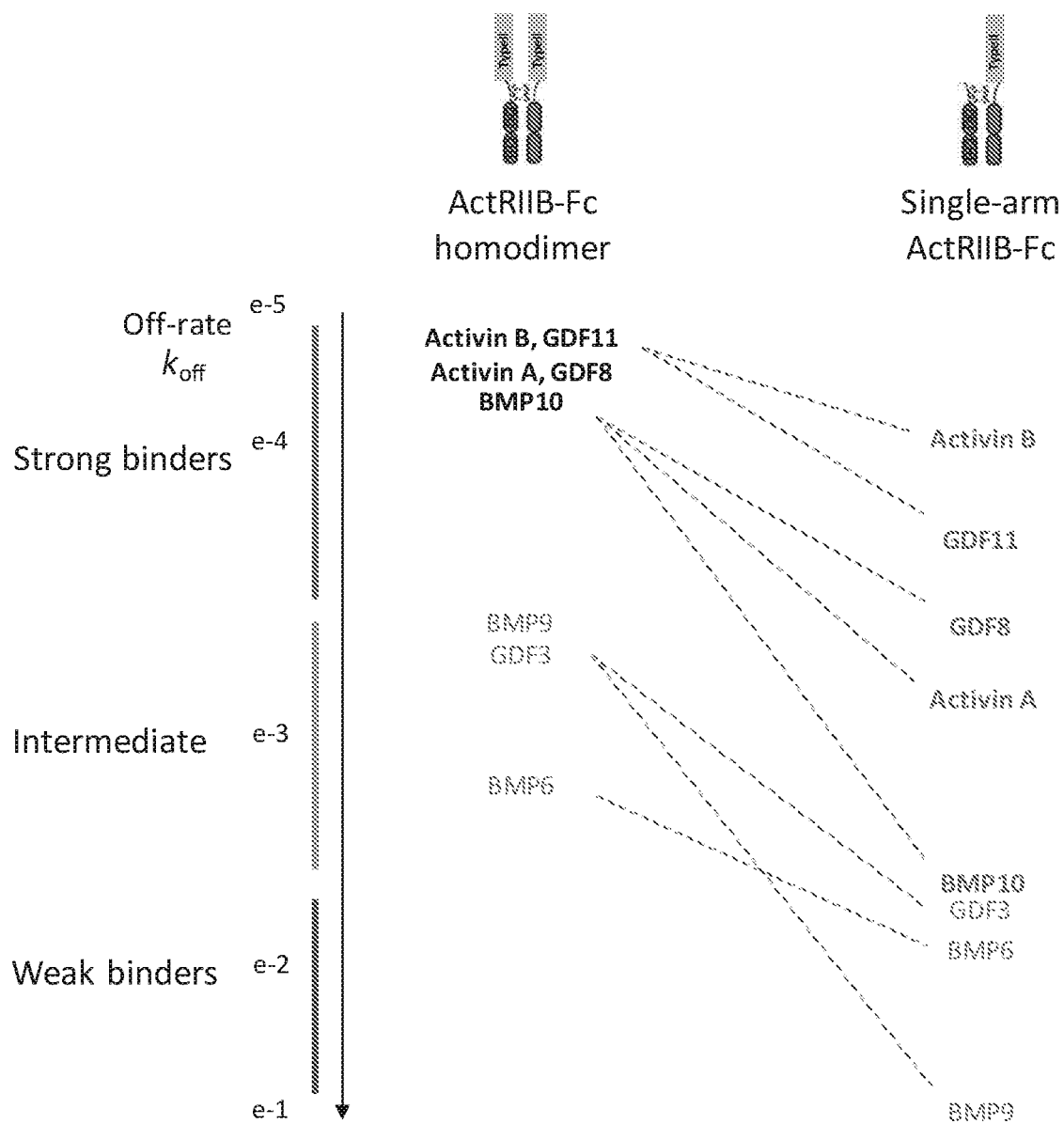


FIGURE 6

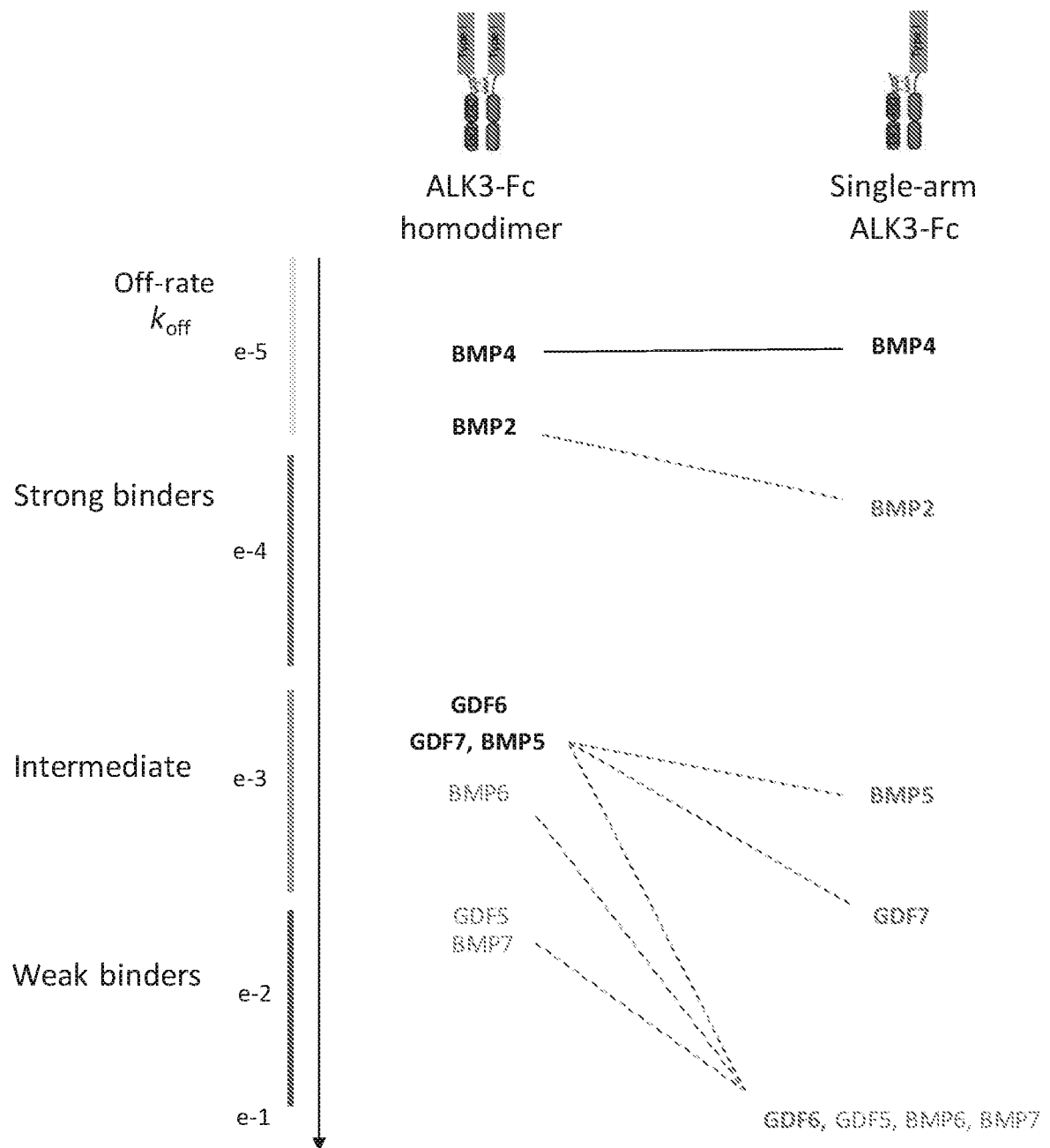


FIGURE 7

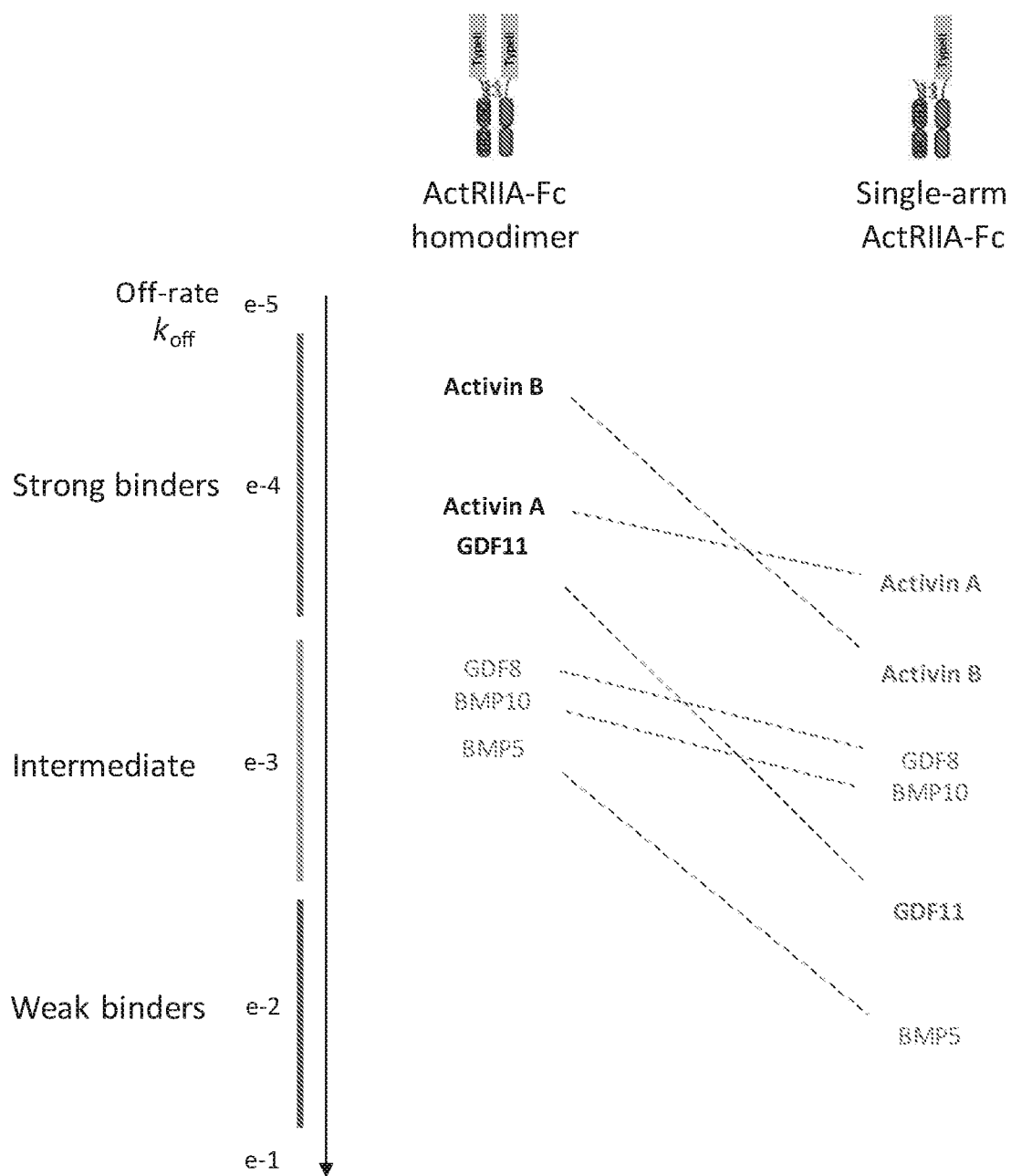


FIGURE 8