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(54) Title: TGF-BETA SUPERFAMILY TYPE I AND TYPE II RECEPTOR HETEROMULTIMERS AND USES THEREOF

(57) Abstract: In certain aspects, the disclosure provides soluble heteromeric polypeptide complexes comprising an extracellular domain of a type I serine/threonine kinase receptor of the TGF-beta family and an extracellular domain of a type II serine/threonine kinase receptor of the TGF-beta family. In some embodiments, the disclosure provides soluble polypeptide complexes comprising an extracellular domain of a type II receptor selected from: ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII. In some embodiments, the disclosure provides soluble polypeptide complexes comprising an extracellular domain of a type I receptor selected from: ALK1, ALK.2, ALK3, ALK4, ALK5, ALK.6, and ALK7. Optionally the soluble complex is a heterodimer. In certain aspects, such soluble polypeptide complexes may be used to regulate (promote or inhibit) growth of tissues or cells including, for example, muscle, bone, cartilage, fat, neural tissue, tumors, cancerous cells, and/or cells of hematopoietic lineages, including red blood cells. In certain aspects, such soluble polypeptide complexes are can be used to improve muscle formation, bone formation, hematopoiesis, metabolic parameters, and disorders associated with these tissues, cellular networks, and endocrine systems.

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

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to United States provisional application serial number 62/143,579, filed April 6, 2015. The disclosure of the foregoing application is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

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

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

SUMMARY OF THE INVENTION

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

Heteromultimeric structures include, for example, heterodimers, heterotrimers, and higher order complexes. See, e.g., Figures 1, 2, 15, 16, 17, 18, 19. In some embodiments heteromultimers of the disclosure are heterodimers. Preferably, TGF-beta superfamily type I and type II receptor polypeptides as described herein comprise a ligand-binding domain of the receptor, for example, an extracellular domain of a TGF-beta superfamily type I or type II receptor. Accordingly, in certain aspects, protein complexes described herein comprise an extracellular domain of a type II TGF-beta superfamily receptor selected from: ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII, as well as truncations and variants thereof, and an extracellular domain of a type I TGF-beta superfamily receptor selected from: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7, as well as truncations and variants thereof. Preferably, TGF-beta superfamily type I and type II polypeptides as described herein, as well as protein complexes comprising the same, are soluble. In certain aspects, heteromultimer 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-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, Müllerian-inhibiting substance (MIS), and Lefty). Optionally, protein complexes of the disclosure bind to one or more of these ligands with a K_D of greater than or equal to 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, or 10^{-12}. In general, heteromultimers of the disclosure antagonize (inhibit) one or more activities of at least one TGF-beta superfamily ligand, and such alterations in activity may be measured using various assays known in the art, including, for example, a cell-based assay as described herein. Preferably heteromultimers of the disclosure exhibit a serum half-life of at least 4, 6, 12, 24, 36, 48, or 72 hours in a mammal (*e.g.*, a mouse or a human). Optionally, heteromultimers of the disclosure may exhibit a serum half-life of at least 6, 8, 10, 12, 14, 20, 25, or 30 days in a mammal (*e.g.*, a mouse or a human).

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

polypeptide and the amino acid sequence of the first member of the interaction pair. Similarly, the TGF-beta superfamily type II receptor polypeptide may be connected directly to the second member of the interaction pair, or an intervening sequence, such as a linker, may be positioned between the amino acid sequence of the TGF-beta superfamily type II receptor polypeptide and the amino acid sequence of the second member of the interaction pair. Examples of linkers include, but are not limited to, the sequences TGGG (SEQ ID NO: 62), TGGGG (SEQ ID NO: 60), SGGGG (SEQ ID NO: 61), GGGG (SEQ ID NO: 59), and GGG (SEQ ID NO: 58).

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

In some embodiments, TGF-beta superfamily type I receptor polypeptides are fusion proteins that comprise an Fc domain of an immunoglobulin. Similarly, in some embodiments, TGF-beta superfamily type II receptor polypeptides are fusion proteins that comprise an Fc domain of an immunoglobulin. Traditional Fc fusion proteins and antibodies are examples of unguided interaction pairs, whereas a variety of engineered Fc domains have been designed as asymmetric interaction pairs [Spiess et al (2015) Molecular Immunology 67(2A): 95-106].

Therefore, a first member and/or a second member of an interaction pair described herein may comprise a constant domain of an immunoglobulin, including, for example, the Fc portion of an immunoglobulin. For example, a first member of an interaction pair may comprise an amino acid sequence that is derived from an Fc domain of an IgG (IgG1, IgG2, IgG3, or IgG4), IgA (IgA1 or IgA2), IgE, or IgM immunoglobulin. 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 comprise an amino acid sequence that is derived from an Fc domain of an IgG (IgG1, IgG2, IgG3, or IgG4), IgA (IgA1 or IgA2), IgE, or IgM. Such immunoglobulin domains may comprise one or more amino acid modifications (e.g., deletions, additions, and/or substitutions) that promote heterodimer formation. For example, the second member of an interaction pair may comprise, consist essentially of, or consist of an amino acid sequence that is at least 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 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. Similarly, 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, including, for example, a modified Fc portion of an immunoglobulin. For example, protein complexes of the disclosure may comprise a first modified Fc portion of an immunoglobulin 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 modified Fc portion of an immunoglobulin, which may be the same or different from the amino acid sequence of the first modified Fc portion of the immunoglobulin, 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. Such immunoglobulin domains may comprise one or more amino acid modifications (e.g., deletions, additions, and/or substitutions) that promote heterodimer formation.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I and type II TGF-beta superfamily receptor polypeptide, wherein the type

II TGF-beta superfamily receptor polypeptide is derived from an ActRIIA receptor. In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type II TGF-beta superfamily receptor polypeptide, wherein at least one of the type II 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, 118, 120, 151, 152, 409, 410, 451, and 452). 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. In certain aspects, heteromeric complexes that comprise an ActRIIA polypeptide further comprise at least one type I TGF-beta superfamily receptor polypeptide. For example, an ActRIIA heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. Optionally, ALK1 polypeptides in this and other embodiments 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. In some embodiments, an ActRIIA heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence

that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. Optionally, ALK2 polypeptides in this and other embodiments 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. In some embodiments, an ActRIIA heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. Optionally, in this and other embodiments 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. In some embodiments, an ActRIIA heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. Optionally, in this and other embodiments 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 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. In some embodiments, an ActRIIA heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or

consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. Optionally, in this and other embodiments 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. In some embodiments, an ActRIIA heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. Optionally, in this and other embodiments 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, in this and other embodiments 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. In some embodiments, an ActRIIA heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476. Optionally, in this and other embodiments, 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). In certain aspects, heteromeric complexes that comprise an ActRIIA polypeptide further comprise at least one different type II TGF-beta superfamily receptor polypeptide. For example, an ActRIIA heteromeric complex may further comprise an ActRIIB polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In some embodiments, an ActRIIA heteromeric complex may further comprise an BMPRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456. In some embodiments, an ActRIIA heteromeric complex may further comprise an MISRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458. In some embodiments, an ActRIIA heteromeric complex may further comprise an TGFBRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I and type II TGF-beta superfamily receptor polypeptide, wherein the type II TGF-beta superfamily receptor polypeptide is derived from an ActRIIB receptor. In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type II TGF-beta superfamily receptor polypeptide, wherein at least one of the type II 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, 100, 102, 153, 154, 401, 402, 453, and 454). 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. Preferably, heteromeric complexes that comprise an ActRIIB polypeptide further comprise at least one type I TGF-beta superfamily receptor polypeptide. For example, an ActRIIB heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some embodiments, an ActRIIB heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, an ActRIIB heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, an ActRIIB heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, an

ActRIIB heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, an ActRIIB heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, an ActRIIB heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476. In certain aspects, heteromeric complexes that comprise an ActRIIB polypeptide further comprise at least one different type II TGF-beta superfamily receptor polypeptide. For example, an ActRIIA heteromeric complex may further comprise an ActRIIB polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. For example, an ActRIIB heteromeric complex may further comprise an ActRIIA polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452. In some embodiments, an ActRIIB heteromeric complex may further comprise an BMPRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456. In some embodiments, an ActRIIB heteromeric complex may further comprise an MISRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and

458. In some embodiments, an ActRIIB heteromeric complex may further comprise an TGFBRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I and type II TGF-beta superfamily receptor polypeptide, wherein the type II TGF-beta superfamily receptor polypeptide is derived from a TGFBRII receptor. In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type II TGF-beta superfamily receptor polypeptide, wherein at least one of the type II TGF-beta superfamily receptor polypeptide is derived from an TGFBRII receptor. For example, TGFBRII 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 TGFBRII sequence disclosed herein (*e.g.*, SEQ ID NOs: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462). Optionally, TGFBRII 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 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, TGFBRII 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 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, TGFBRII polypeptides of the disclosure may be fusion proteins that further comprise one or more portions (domains) that are heterologous to TGFBRII. For example, a TGFBRII polypeptide may be fused to a heterologous polypeptide that comprises a multimerization domain, optionally with a linker domain positioned between the TGFBRII polypeptide and the heterologous polypeptide. In some embodiments, multimerization

domains described herein comprise one component of an interaction pair. Preferably, heteromeric complexes that comprise a TGFBRII polypeptide further comprise at least one type I TGF-beta superfamily receptor polypeptide. For example, a TGFBRII heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some embodiments, a TGFBRII heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, a TGFBRII heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, a TGFBRII heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, a TGFBRII heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, a TGFBRII heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, a TGFBRII heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306,

309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476. Optionally, heteromeric complexes comprising a TGFBRII polypeptide may further comprise one or more additional type II TGF-beta superfamily receptor polypeptides, including, for example ActRIIA, ActRIIB, BMPRII, and MISRII polypeptides described herein (*e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 9, 10, 11 46, 47, 50, 51, 71, 72, 75, 76, 79, 80, 100, 102, 118, 120, 121, 123, 401, 402, 409, 410, 411, and 412). In certain aspects, heteromeric complexes that comprise an TGFBRII polypeptide further comprise at least one different type II TGF-beta superfamily receptor polypeptide. For example, an TGFBRII heteromeric complex may further comprise an ActRIIA polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452. In some embodiments, a TGFBRII heteromeric complex may further comprise an ActRIIB polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In some embodiments, a TGFBRII heteromeric complex may further comprise an MISRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458. In some embodiments, a TGFBRII heteromeric complex may further comprise an BMPRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I and type II TGF-beta superfamily receptor polypeptide, wherein the type II TGF-beta superfamily receptor polypeptide is derived from a BMPRII receptor. In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type II TGF-beta superfamily receptor polypeptide, wherein at least one of the type II TGF-beta superfamily receptor polypeptide is derived from an 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 BMPRII sequence disclosed herein (*e.g.*, SEQ ID NOs: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456). 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 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 that comprises a multimerization domain, optionally with a linker domain positioned between the BMPRII polypeptide and the heterologous polypeptide. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. Preferably, heteromeric complexes that comprise a BMPRII polypeptide further comprise at least one type I TGF-beta superfamily receptor polypeptide. For example, a BMPRII heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some embodiments, a BMPRII heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, a BMPRII heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, a BMPRII heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a

sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, a BMPRII heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, a BMPRII heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, a BMPRII heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476. In certain aspects, heteromeric complexes that comprise an BMPRII polypeptide further comprise at least one different type II TGF-beta superfamily receptor polypeptide. For example, an BMPRII heteromeric complex may further comprise an ActRIIA polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452. In some embodiments, a BMPRII heteromeric complex may further comprise an ActRIIB polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In some embodiments, a BMPRII heteromeric complex may further comprise an MISRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458. In some embodiments, a BMPRII heteromeric complex may further comprise an TGFBRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%,

95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising a type I and type II TGF-beta superfamily receptor polypeptide, wherein the type II TGF-beta superfamily receptor polypeptide is derived from an MISRII receptor. In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type II TGF-beta superfamily receptor polypeptide, wherein at least one of the type II TGF-beta superfamily receptor polypeptide is derived from an MISRII receptor. In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type II TGF-beta superfamily receptor polypeptide, wherein at least one of the type II 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 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, 133, 135, 161, 162, 419, 420, 457, and 458). 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. Preferably, heteromeric complexes that comprise an MISRII polypeptide further comprise at least one type I TGF-beta superfamily polypeptide. For example, an MISRII heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some

embodiments, an MISRII heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, an MISRII heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, an MISRII heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, an MISRII heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, an MISRII heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, an MISRII heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476. In certain aspects, heteromeric complexes that comprise an MISRII polypeptide further comprise at least one different type II TGF-beta superfamily receptor polypeptide. For example, an MISRII heteromeric complex may further comprise an ActRIIA polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452. In some embodiments,

an MISRII heteromeric complex may further comprise an ActRIIB polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In some embodiments, an MISRII heteromeric complex may further comprise an BMPRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456. In some embodiments, an MISRII heteromeric complex may further comprise an TGFBRII polypeptide as described herein, including, for example, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type I TGF-beta superfamily receptor polypeptide, wherein at least one of the type I 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.*, 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464). 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. In some embodiments, heteromeric complexes that comprise an ALK1 polypeptide further comprise

at least one different type I TGF-beta superfamily polypeptide. For example, an ALK1 heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, an ALK1 heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, an ALK1 heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, an ALK1 heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, an ALK1 heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, an ALK1 heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type I TGF-beta superfamily receptor polypeptide, wherein at least one of the type I 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.,* 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466). 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. In some embodiments, heteromeric complexes that comprise an ALK2 polypeptide further comprise at least one different type I TGF-beta superfamily polypeptide. For example, an ALK2 heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some embodiments, an ALK2 heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, an ALK2 heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, an ALK2 heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, an ALK2 heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, *e.g.*, a

polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, an ALK2 heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type I TGF-beta superfamily receptor polypeptide, wherein at least one of the type I 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.*, 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468). 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 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. In some embodiments, heteromeric complexes that comprise an ALK3 polypeptide further comprise at least one different type I TGF-beta superfamily polypeptide. For example, an ALK3 heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466.

In some embodiments, an ALK3 heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some embodiments, an ALK3 heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, an ALK3 heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, an ALK3 heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, an ALK3 heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type I TGF-beta superfamily receptor polypeptide, wherein at least one of the type I 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 sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an ALK4 sequence disclosed herein (*e.g.*, 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470).). 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 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. 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. In some embodiments, heteromeric complexes that comprise an ALK4 polypeptide further comprise at least one different type I TGF-beta superfamily polypeptide. For example, an ALK4 heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, an ALK4 heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, an ALK4 heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some embodiments, an ALK4 heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, an ALK4 heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, an ALK4 heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, *e.g.*, a

polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type I TGF-beta superfamily receptor polypeptide, wherein at least one of the type I 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.,* 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472). 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. In some embodiments, heteromeric complexes that comprise an ALK5 polypeptide further comprise at least one different type I TGF-beta superfamily polypeptide. For example, an ALK5 heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, an ALK5 heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, an ALK5

heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, an ALK5 heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some embodiments, an ALK5 heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, an ALK5 heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type I TGF-beta superfamily receptor polypeptide, wherein at least one of the type I 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.*, 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474). 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. In some embodiments, heteromeric complexes that comprise an ALK6 polypeptide further comprise at least one different type I TGF-beta superfamily polypeptide. For example, an ALK6 heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, an ALK6 heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, an ALK6 heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, an ALK6 heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, an ALK6 heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence

selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464. In some embodiments, an ALK6 heteromeric complex may further comprise an ALK7 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

In some embodiments, the disclosure provides heteromeric polypeptide complexes comprising at least two different type I TGF-beta superfamily receptor polypeptide, wherein at least one of the type I 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.*, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476). 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. In some embodiments, multimerization domains described herein comprise one component of an interaction pair. In some embodiments, heteromeric complexes that comprise an ALK7 polypeptide further comprise at least one different type I TGF-beta superfamily polypeptide. For example, an ALK7 heteromeric complex may further comprise an ALK2 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466. In some embodiments, an ALK7 heteromeric complex may further comprise an ALK3 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%,

95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468. In some embodiments, an ALK7 heteromeric complex may further comprise an ALK4 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470. In some embodiments, an ALK7 heteromeric complex may further comprise an ALK5 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472. In some embodiments, an ALK7 heteromeric complex may further comprise an ALK6 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474. In some embodiments, an ALK7 heteromeric complex may further comprise an ALK1 polypeptide as described herein, including, *e.g.*, a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a sequence selected from SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464.

In some embodiments, the TGF-beta superfamily type I and/or type II receptor polypeptides disclosed herein comprise one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent. In some embodiments, the TGF-beta superfamily type I and/or type II polypeptides described herein are glycosylated and have a glycosylation pattern obtainable from the expression of the polypeptides in a mammalian cell, including, for example, a CHO cell.

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

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

Any of the protein complexes described herein may be incorporated into a pharmaceutical preparation. Optionally, such pharmaceutical preparations are at least 80%, 85%, 90%, 95%, 97%, 98% or 99% pure with respect to other polypeptide components. Optionally, pharmaceutical preparations disclosed herein may comprise one or more additional active agents. In some embodiments, heteromultimers of the disclosure comprise less than 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% type I receptor polypeptide homomultimers. In some embodiments, heteromultimers of the disclosure comprise less than 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% type II receptor polypeptide homomultimers. In some embodiments, heteromultimers of the disclosure comprise less than 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% type I receptor polypeptide

homomultimers and less than 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% type II receptor polypeptide homomultimers.

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

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show two schematic examples of heteromeric protein complexes comprising type I receptor and type II receptor polypeptides. Figure 1A depicts a heterodimeric protein complex comprising one type I receptor fusion polypeptide and one type II receptor fusion polypeptide, which can be assembled covalently or noncovalently via a multimerization domain contained within each polypeptide chain. Two assembled multimerization domains constitute an interaction pair, which can be either guided or unguided. Figure 2A depicts a heterotetrameric protein complex comprising two heterodimeric complexes as in Figure 1A. Complexes of higher order can be envisioned.

Figure 2 shows a schematic example of a heteromeric protein complex comprising a type I receptor polypeptide (indicated as "I") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6 or ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, 464, 18, 19, 136, 138, 173, 174, 421, 422, 465, 466, 22, 23, 115, 117, 175, 176, 407, 408, 467, 468, 26, 27,

83, 84, 104, 106, 177, 178, 403, 404, 469, 470, 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, 472, 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, 474, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476) and a type II receptor polypeptide (indicated as "II") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIA, ActRIIB, MISRII, BMPRII, or TGFBRII protein from humans or other species such as those described herein, e.g., 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, 452, 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, 454, 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, 456, 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, 458, 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462). In the illustrated embodiment, the type I receptor polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C"), and the type II receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("D"). In each fusion polypeptide, a linker may be positioned between the type I or type II receptor polypeptide and the corresponding member of the interaction pair. The first and second members of the interaction pair (C, D) may be a guided (asymmetric) pair, meaning that the members of the pair associate preferentially with each other rather than self-associate, or the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and may have the same or different amino acid sequences. Traditional Fc fusion proteins and antibodies are examples of unguided interaction pairs, whereas a variety of engineered Fc domains have been designed as guided (asymmetric) interaction pairs [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106].

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

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

Figure 5 shows multiple sequence alignment of Fc domains from human IgG isotypes using Clustal 2.1. Hinge regions are indicated by dotted underline. Double underline

indicates examples of positions engineered in IgG1 Fc to promote asymmetric chain pairing and the corresponding positions with respect to other isotypes IgG2, IgG3 and IgG4.

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

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

Figure 8 shows ligand binding data for an ActRIIB-Fc:ALK7-Fc heterodimeric protein complex as compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer. Format is the same as in Figure 6. As shown, four of the five ligands with strong binding to ActRIIB-Fc homodimer (activin A, BMP10, GDF8, and GDF11) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer, the exception being activin B which retains tight binding to the heterodimer. In addition, three ligands with intermediate binding to ActRIIB-Fc homodimer (GDF3, BMP6, and particularly BMP9) exhibit reduced binding to the

ActRIIB-Fc:ALK7-Fc heterodimer. In contrast, BMP5 binds the ActRIIB-Fc:ALK7 heterodimer with intermediate strength despite only weak binding to ActRIIB-Fc homodimer. No ligands tested bind to ALK7-Fc homodimer.

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

Figure 10 shows ligand binding data for an ActRIIA-Fc:ALK4-Fc heterodimeric protein complex as compared to ActRIIA-Fc homodimer and ALK4-Fc homodimer. Format is the same as in Figure 6. As shown, the ActRIIA-Fc:ALK4-Fc heterodimer exhibits enhanced binding to activin A, and particularly enhanced binding to activin AC, compared to ActRIIA-Fc homodimer, while retaining strong binding to activin AB and GDF11. In addition, the ligand with highest affinity for ActRIIA-Fc homodimer, activin B, displays reduced affinity (albeit still within the high-affinity range) for the ActRIIA-Fc:ALK4-Fc heterodimer. The ActRIIA-Fc:ALK4-Fc heterodimer also exhibits markedly reduced binding to BMP10 compared to ActRIIA-Fc homodimer.

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

Figure 12 shows ligand binding data for a BMPRII-Fc:ALK3-Fc heterodimeric protein complex as compared to BMPRII-Fc homodimer and ALK3-Fc homodimer. Format is the same as in Figure 6. As shown, the BMPRII-Fc:ALK3-Fc heterodimer binds much more strongly to BMP6 than does ALK3-Fc homodimer, reflecting an off-rate nearly ten times slower. With its largely unchanged binding to BMP2 and BMP4, the BMPRII-

Fc:ALK3 heterodimer can therefore be considered a joint inhibitor of BMP2, BMP4, and BMP6. This binding profile contrasts with that of ALK3-Fc homodimer, whose exceptionally strongly binding to BMP4 and BMP2 identifies it as highly selective for this ligand pair compared to four ligands with intermediate-level binding, including BMP6.

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

Figure 14 shows ligand binding data for two different TGFβRII-Fc:ALK5-Fc heterodimeric protein complexes as compared to TGFβRII-Fc homodimer and ALK5-Fc homodimer. Format is the same as in Figure 6. As shown, TGFβRII-Fc:ALK5-Fc heterodimers differ markedly from TGFβRII-Fc homodimer in their high selectivity for TGFβ2 while still retaining considerable affinity for TGFβ1 and TGFβ3. The heterodimer incorporating the long isoform of TGFβRII bound TGFβ2 more strongly and selectively than did its short-isoform counterpart. No ligands tested bind to ALK5-Fc homodimer.

Figures 15A-15D show schematic examples of heteromeric protein complexes comprising a type I receptor polypeptide (indicated as "I") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6 or ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, 464, 18, 19, 136, 138, 173, 174, 421, 422, 465, 466, 22, 23, 115, 117, 175, 176, 407, 408, 467, 468, 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, 470, 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, 472, 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, 474, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476) and a type II receptor polypeptide (indicated as "II") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIA, ActRIIB, MISRII, BMPRII, or TGFBRII protein from humans or other species such as those described herein, e.g., 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, 452, 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, 454, 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, 456, 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, 458, 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160,

415, 416, 417, 418, 459, 460, 461, and 462). In the illustrated embodiments, the a type I receptor polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"), and a type II receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"). Suitable interaction pairs included, for example, heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof such as those described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. In each fusion polypeptide, a linker may be positioned between the a type I receptor polypeptide or a type II receptor polypeptide and the corresponding member of the interaction pair. The first and second members of the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference, and they may have the same or different amino acid sequences. See Figure 15A. Alternatively, the interaction pair may be a guided (asymmetric) pair, meaning that the members of the pair associate preferentially with each other rather than self-associate. See Figure 15B. Complexes of higher order can be envisioned. See Figure 15C and 15D.

Figures 16A-16G show schematic examples of heteromeric protein complexes comprising two type I receptor polypeptide (indicated as "I") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6 or ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, 464, 18, 19, 136, 138, 173, 174, 421, 422, 465, 466, 22, 23, 115, 117, 175, 176, 407, 408, 467, 468, 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, 470, 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, 472, 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, 474, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476) and two type II receptor polypeptide (indicated as "II") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIA, ActRIIB, MISRII, BMPRII, or TGFBRII protein from humans or other species such as those described herein, e.g., 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, 452, 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, 454, 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, 456, 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, 458, 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462).

In the illustrated embodiment 16A, the first type I receptor polypeptide (from left to right) is part of a fusion polypeptide that comprises a first member of an interaction pair

("C_1") and further comprises an additional first member of an interaction pair ("A_1"); and the second type I receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2") and further comprises an first member of an interaction pair ("A_2"). The first type II receptor polypeptide (from left to right) is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_1"); and the second type II receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_2"). A_1 and A_2 may be the same or different; B_1 and B_2 may be the same or different, and C_1 and C_2 may be the same or different. In each fusion polypeptide, a linker may be positioned between the type I receptor polypeptide or type II receptor polypeptide and the corresponding member of the interaction pair as well as between interaction pairs. Figure 9A is an example of an association of unguided interaction pairs, 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.

In the illustrated embodiment 16B, the first type II receptor polypeptide (from left to right) is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1") and further comprises an additional first member of an interaction pair ("A_1"); and the second type II receptor ActRIIB polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_2"). The first type I receptor polypeptide (from left to right) is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_1"); and the second type I receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2") and further comprises a first member of an interaction pair ("A_2"). In each fusion polypeptide, a linker may be positioned between the type I receptor or type II receptor polypeptide and the corresponding member of the interaction pair as well as between interaction pairs. Figure 16B is an example of an association of guided (asymmetric) interaction pairs, meaning that the members of the pair associate preferentially with each other rather than self-associate.

Suitable interaction pairs included, for example, heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof as described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Complexes of higher order can be envisioned. See Figure 16C-16F. Using similar methods, particularly those that employ light and/or heavy chain immunoglobulins, truncations, or variants thereof, interaction pairs may be used to produce heterodimers that resemble antibody Fab and $F(ab')_2$ complexes [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. See Figure 16G.

Figures 17A and 17B show schematic examples of a heteromeric protein complex comprising a type I receptor polypeptide (indicated as "I") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK1, ALK2, ALK3, ALK4, ALK5, ALK6 or ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, 464, 18, 19, 136, 138, 173, 174, 421, 422, 465, 466, 22, 23, 115, 117, 175, 176, 407, 408, 467, 468, 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, 470, 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, 472, 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, 474, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476) and a type II receptor polypeptide (indicated as "II") (e.g. a polypeptide that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIA, ActRIIB, MISRII, BMPRII, or TGFBRII protein from humans or other species such as those described herein, e.g., 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, 452, 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, 454, 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, 456, 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, 458, 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462), and a ligand-binding domain of an antibody (e.g., a ligand binding domain derived form an antibody that binds to one or more TGF-beta superfamily ligands). In the illustrated embodiments, the type I receptor polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"), and further comprises an additional first member of an interaction pair ("A_1"). The type II receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_1"). The variable heavy chain (V_H) polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"), and further comprises a first member of an interaction pair ("A_2"). The variable heavy chain (V_L) polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_2"). In each fusion polypeptide, a linker may be positioned between the type I receptor or type II receptor polypeptide and the corresponding member of the interaction pair, between interaction pairs, and between the V_H and V_L polypeptides and a member of the interaction pair. A_1 and A_2 may be the same or different; B_1 and B_2 may be the same or different, and C_1 and C_2 may be the same or different. Suitable interaction pairs included, for example, constant heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof as described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Figure 17A is an example of an association of guided (asymmetric) interaction pairs, meaning that the members of the pair associate preferentially

with each other rather than self-associate. Figure 17B is an example of an association of unguided interaction pairs, 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.

Figure 18 shows schematic examples of type I receptor: type II receptor single-trap polypeptides. Type I receptor: type II receptor single-trap polypeptides may contain multiple type I receptor domains (e.g., 1, 2, 3, 4, 5, 6, 7, 9, 10 or more domains), having the same or different sequences, and multiple type II receptor domains (e.g., 1, 2, 3, 4, 5, 6, 7, 9, 10 or more domains), having the same or different sequences. These type I receptor and type II receptor domains may be arranged in any order and may comprise one or more linker domains positions between one or more of the type 11 receptor and type II receptor domains. Such ligand traps may be useful as therapeutic agents to treat or prevent diseases or disorders described herein.

Figure 19A-19D show schematic examples of multimeric protein complexes comprising at least one type I receptor: type II receptor single-chain trap polypeptide. In the illustrated embodiments 19A and 19B, a first type I receptor: type II receptor single-chain trap polypeptide (from left to right) is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"); and a second type I receptor: type II receptor single-chain trap polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"). C_1 and C_2 may be the same or different. The first and second type I receptor: type II receptor single-chain trap polypeptides may be the same or different. In each fusion polypeptide, a linker may be positioned between the type I receptor: type II receptor single-chain trap polypeptide and the corresponding member of the interaction pair. Suitable interaction pairs included, for example, heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof as described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Figure 19A is an example of an association of unguided interaction pairs, 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. Figure 129 is an example of an association of guided (asymmetric) interaction pairs, meaning that the members of the pair associate preferentially with each other rather than self-associate. Complexes of higher order can be envisioned. In addition, such type I receptor: type II receptor single-chain trap polypeptides may be similarly be associated, covalently or non-covalently, with one or more type I receptor

polypeptides and/or one or more type II receptor polypeptides. See Figure 19C. Also, such type I receptor: type II receptor single-chain trap polypeptides may be similarly be associated, covalently or non-covalently, with one or more ligand-binding domain of an antibody (e.g., a ligand binding domain of an antibody that binds to one or more type I receptor: type II receptor heteromultimer binding-ligands). See Figure 19D.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

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

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

aberrant TGF-beta superfamily signaling is associated with a wide range of human pathologies including, for example, autoimmune disease, cardiovascular disease, fibrotic disease, and cancer.

Ligands of the TGF-beta superfamily share the same dimeric structure in which the central 3-1/2 turn helix of one monomer packs against the concave surface formed by the beta-strands of the other monomer. The majority of TGF-beta family members are further stabilized by an intermolecular disulfide bonds. This disulfide bond traverses through a ring formed by two other disulfide bonds generating what has been termed a 'cysteine knot' motif. See, *e.g.*, Lin et al., (2006) Reproduction 132: 179-190 and Hinck et al. (2012) FEBS Letters 586: 1860-1870.

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

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

TGF-beta isoforms are the founding members of the TGF-beta superfamily, of which there are 3 known isoforms in mammals designated as TGF-beta1, TGF-beta2 and TGF-beta3. Mature bioactive TGF-beta ligands function as homodimers and predominantly signal through the type I receptor ALK5, but have also been found to additionally signal through

ALK1 in endothelial cells. See, *e.g.*, Goumans *et al.* (2003) Mol Cell 12(4): 817-828. TGF-beta1 is the most abundant and ubiquitously expressed isoform. TGF-beta1 is known to have an important role in wound healing, and mice expressing a constitutively active TGF-beta1 transgene develop fibrosis. See *e.g.*, Clouthier *et al.*, (1997) J Clin. Invest. 100(11): 2697-2713. TGF-beta1 is also involved in T cell activation and maintenance of T regulatory cells. See, *e.g.*, Li *et al.*, (2006) Immunity 25(3): 455-471. TGF-beta2 expression was first described in human glioblastoma cells, and is occurs in neurons and astroglial cells of the embryonic nervous system. TGF-beta2 is known to suppress interleukin-2-dependent growth of T lymphocytes. TGF-beta3 was initially isolated from a human rhabdomyosarcoma cell line and since has been found in lung adenocarcinoma and kidney carcinoma cell lines. TGF-beta3 is known to be important for palate and lung morphogenesis. See, *e.g.*, Kubiczkova et al., (2012) Journal of Translational Medicine 10:183.

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

As described herein, agents that bind to "activin A" are agents that specifically bind to the β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (*e.g.*, a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of a heterodimer complex (*e.g.*, a $\beta_A\beta_B$ heterodimer), agents that bind to "activin A" are specific for epitopes present within the

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

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

The BMPs and GDFs together form a family of cysteine-knot cytokines sharing the characteristic fold of the TGF-beta superfamily. See, *e.g.*, Rider *et al.* (2010) Biochem J., 429(1):1-12. This family includes, for example, BMP2, BMP4, BMP6, BMP7, BMP2a, BMP3, BMP3b (also known as GDF10), BMP4, BMP5, BMP6, BMP7, BMP8, BMP8a, BMP8b, BMP9 (also known as GDF2), BMP10, BMP11 (also known as GDF11), BMP12 (also known as GDF7), BMP13 (also known as GDF6), BMP14 (also known as GDF5), BMP15, GDF1, GDF3 (also known as VGR2), GDF8 (also known as myostatin), GDF9, GDF15, and decapentaplegic. Besides the ability to induce bone formation, which gave the BMPs their name, the BMP/GDFs display morphogenetic activities in the development of a wide range of tissues. BMP/GDF homo- and hetero-dimers interact with combinations of type I and type II receptor dimers to produce multiple possible signaling complexes, leading to the activation of one of two competing sets of SMAD transcription factors. BMP/GDFs have highly specific and localized functions. These are regulated in a number of ways, including the developmental restriction of BMP/GDF expression and through the secretion of several specific BMP antagonist proteins that bind with high affinity to the cytokines. Curiously, a number of these antagonists resemble TGF-beta superfamily ligands.

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

or structurally related proteins and inhibit their biological activity include follistatin, and potentially, follistatin-related proteins. See, *e.g.*, Gamer *et al.* (1999) Dev. Biol., 208: 222-232.

GDF11, also known as BMP11, is a secreted protein that is expressed in the tail bud, limb bud, maxillary and mandibular arches, and dorsal root ganglia during mouse development. See, *e.g.*, McPherron *et al.* (1999) Nat. Genet., 22: 260-264; and Nakashima *et al.* (1999) Mech. Dev., 80: 185-189. GDF11 plays a unique role in patterning both mesodermal and neural tissues. See, *e.g.*, Gamer *et al.* (1999) Dev Biol., 208:222-32. GDF11 was shown to be a negative regulator of chondrogenesis and myogenesis in developing chick limb. See, *e.g.*, Gamer *et al.* (2001) Dev Biol., 229:407-20. The expression of GDF11 in muscle also suggests its role in regulating muscle growth in a similar way to GDF8. In addition, the expression of GDF11 in brain suggests that GDF11 may also possess activities that relate to the function of the nervous system. Interestingly, GDF11 was found to inhibit neurogenesis in the olfactory epithelium. See, *e.g.*, Wu *et al.* (2003) Neuron., 37:197-207. Hence, GDF11 may have in vitro and in vivo applications in the treatment of diseases such as muscle diseases and neurodegenerative diseases (*e.g.*, amyotrophic lateral sclerosis).

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

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

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

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

Activin receptor-like kinase-3 (ALK3), the product of the *BMPR1A* gene known alternatively as ACVRLK3, is a type I receptor mediating effects of multiple ligands in the BMP family. Unlike several type I receptors with ubiquitous tissue expression, ALK3 displays a restricted pattern of expression consistent with more specialized functionality. See, *e.g.*, ten Dijke (1993) Oncogene, 8: 2879-2887 and OMIM entry 601299. ALK3 is generally recognized as a high affinity receptor for BMP2, BMP4, BMP7 and other members of the BMP family. BMP2 and BMP7 are potent stimulators of osteoblastic differentiation, and are now used clinically to induce bone formation in spine fusions and certain non-union fractures. ALK3 is regarded as a key receptor in mediating BMP2 and BMP4 signaling in osteoblasts. See, *e.g.*, Lavery *et al.* (2008) J. Biol. Chem. 283: 20948-20958. A homozygous ALK3 knockout mouse dies early in embryogenesis (~day 9.5), however, adult mice carrying a

conditional disruption of ALK3 in osteoblasts have been recently reported to exhibit increased bone mass, although the newly formed bone showed evidence of disorganization. See, *e.g.*, Kamiya (2008) J. Bone Miner. Res., 23:2007-2017; and Kamiya (2008) Development 135: 3801-3811. This finding is in startling contrast to the effectiveness of BMP2 and BMP7 (ligands for ALK3) as bone building agents in clinical use.

Activin receptor-like kinase-4 (ALK4), the product of the *ACVR1B* gene alternatively known as ACVRLK4, is a type I receptor that transduces signaling for a number of TGF-beta family ligands including activins, nodal and GDFs. ALK4 mutations are associated with pancreatic cancer and expression of dominant negative truncated ALK4 isoforms are highly expressed in human pituitary tumors. See, *e.g.*, Tsuchida *et al.*, (2008) Endocrine Journal 55(1):11-21 and OMIM entry 601300.

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

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

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

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

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

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

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

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

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

polypeptide and an extracellular domain of a TGFβ superfamily type II receptor polypeptide have different ligand binding specificities/profiles in comparison to their corresponding homomultimer complexes.

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

The terms "heteromer" or "heteromultimer" is a complex comprising at least a first polypeptide and a second polypeptide, wherein the second polypeptide differs in amino acid sequence from the first polypeptide by at least one amino acid residue. The heteromer can comprise a "heterodimer" formed by the first and second polypeptide or can form higher order structures where polypeptides in addition to the first and second polypeptide are present. Exemplary structures for the heteromultimer include heterodimers, heterotrimers, heterotetramers and further oligomeric structures. Heterodimers are designated herein as X:Y or equivalently as X-Y, where X represents a first polypeptide and Y represents a second polypeptide. 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 components 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.

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

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

As used herein, unless otherwise stated, "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*", wherein "*X*" is a specified agent such as protein or nucleic acid.

The terms "about" and "approximately" as used in connection with a numerical value throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. In general, such interval of accuracy is ± 10%.

Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably ≤ 5 -fold and more preferably ≤ 2-fold of a given value.

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

The terms "a" and "an" include plural referents unless the context in which the term is used clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein. Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two or more specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

Throughout this specification, the word "comprise" or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or groups of integers but not the exclusion of any other integer or group of integers.

2. TGF-beta Superfamily Type I Receptor and Type II Receptor Complexes

In certain aspects, the present disclosure relates to heteromultimer complexes comprising one or more TGF-beta superfamily type I receptor polypeptides (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7 proteins from humans or other species such as those described herein, e.g., SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, 464, 18, 19, 136, 138, 173, 174, 421, 422, 465, 466, 22, 23, 115, 117, 175, 176, 407, 408, 467, 468, 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, 470, 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, 472, 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, 474, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476) and one or more TGF-beta superfamily type II receptor polypeptides (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII proteins from humans or other species such as those described herein, e.g., SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, 452, 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, 454, 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, 456, 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, 458, 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462); heteromultimer complexes comprising at least two different TGF-beta superfamily type I receptor polypeptides (*e.g.*, ALK1, ALK2,

ALK3, ALK4, ALK5, ALK6, and ALK7 proteins from humans or other species such as those described herein, e.g., SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, 464, 18, 19, 136, 138, 173, 174, 421, 422, 465, 466, 22, 23, 115, 117, 175, 176, 407, 408, 467, 468, 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, 470, 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, 472, 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, 474, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476); and heteromultimer complexes comprising at least two different TGF-beta superfamily type II receptor polypeptides (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII proteins from humans or other species such as those described herein, e.g., SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, 452, 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, 454, 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, 456, 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, 458, 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462), which are generally referred to herein as "heteromultimer complexes" or "heteromultimers". Preferably, heteromultimers are soluble, *e.g.*, a heteromultimer comprises a soluble portion of at least one TGFβ superfamily type I receptor polypeptide and a soluble portion (domain) of at least one TGFβ superfamily type II receptor polypeptide. In general, the extracellular domains of TGFβ superfamily type I and type II receptors correspond to a soluble portion of the type I and type II receptor. Therefore, in some embodiments, heteromultimers of the disclosure comprise an extracellular domain of a TGFβ superfamily type I receptor polypeptide (*e.g.*, one or more ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and/or ALK7 receptor extracellular domains) and/or an extracellular domain of a TGFβ superfamily type II receptor polypeptide (*e.g.*, one or more ActRIIA, ActRIIB, TGFBRII, BMPRII, and/or MISRII receptor extracellular domains). Exemplary extracellular domains of ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII are disclosed herein and such sequences, as well as fragments, functional variants, and modified forms thereof, may be used in accordance with the inventions of the present disclosure (*e.g.*, heteromultimers compositions and uses thereof). Heteromultimers of the disclosure include, e.g., heterodimers, heterotrimers, heterotetramers, and higher order oligomeric structures. See, e.g., Figures 1, 2, and 15-17. In certain preferred embodiments, heteromultimers of the disclosure are heterodimers.

A defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by 10, 12, or 14 conserved cysteine residues located at varying positions within the extracellular domain of each monomeric

receptor. See, *e.g.*, Greenwald et al. (1999) Nat Struct Biol 6:18-22; Hinck (2012) FEBS Lett 586:1860-1870. The core ligand-binding domains of TGFβ superfamily receptors, as demarcated by the outermost of these conserved cysteines, corresponds to positions 29-109 of SEQ ID NO: 1 (ActRIIB precursor); positions 30-110 of SEQ ID NO: 9 (ActRIIA precursor); positions 34-95 of SEQ ID NO: 14 (ALK1 precursor); positions 35-99 of SEQ ID NO: 18 (ALK2 precursor); positions 61-130 of SEQ ID NO: 22 (ALK3 precursor); positions 34-101 of SEQ ID NOs: 26 and 83 (ALK4 precursors); positions 36-106 of SEQ ID NOs: 30 and 87 (ALK5 precursors); positions 32-102 of SEQ ID NO: 34 (ALK6 isoform B precursor); positions 28-92 of SEQ ID NOs: 38, 305, and 309 (ALK7 precursors); positions 51-143 of SEQ ID NO: 42 (TGFBRII isoform B precursor); positions 34-123 of SEQ ID NO: 46 and 71 (BMPRII precursors); positions 24-116 of SEQ ID NO: 50, 75, and 79 (MISRII precursors); positions 44-168 of SEQ ID NO: 67 (TGFBRII isoform A precursor); and positions 62-132 of SEQ ID NO: 91 (ALK6 isoform A precursor). The structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated 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 preferred embodiments, heteromultimers of the disclosure bind to and/or inhibit (antagonize) activity of one or more TGF-beta superfamily ligands including, but not limited to, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty. In particular, heteromultimer complexes of the disclosure may be used to antagonize signaling transduction (*e.g.*, Smad 2/3 and/or Smad 1/5/8 signaling) initiated by one or more TGFβ superfamily ligands, which may be determined, for example, using a cell-based assay such as those described herein. As described herein, such antagonist heteromultimer complexes may be useful in the treatment or prevention of various disorders/conditions associated with, *e.g.*, muscle loss, insufficient muscle growth, neurodegeneration, bone loss, reduced bone density and/or mineralization, insufficient bone growth, and/or obesity. In some embodiments, heteromultimer complexes

of the disclosure have different ligand binding specificities/profiles in comparison to their corresponding homomultimer complex (*e.g.*, an ALK4:ActRIIB heterodimer vs. a corresponding ActRIIB or ALK4 homodimer).

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

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

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

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 (mature) extracellular ActRIIB polypeptide sequence is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDD FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 2).

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

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDD FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO: 3).

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

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 GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
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 (mature) extracellular ActRIIB polypeptide sequence of the alternative A64 form is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDD FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 5)

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

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDD FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO: 6)

A nucleic acid sequence encoding the human ActRIIB precursor protein is shown below (SEQ ID NO: 7), representing nucleotides 25-1560 of Genbank Reference Sequence NM_001106.3, which encode amino acids 1-513 of the ActRIIB precursor. The sequence as shown 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
  51 CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
 101 CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA
 151 GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
 201 TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
 251 GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
 301 TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC
 351 AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
 401 CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC
 451 CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA
 501 CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC
 551 TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
 601 TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA
 651 GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT
 701 TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC
 751 GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT
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 801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951 TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA
1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101 TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201 AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251 GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA
1301 AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351 GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC
1401 TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC
1501 ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC (SEQ ID NO: 7)
```

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.

```
   1 GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
  51 GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
 101 AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
 151 ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
 201 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
 251 GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
 301 GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
(SEQ ID NO: 8)
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An alignment of the amino acid sequences of human ActRIIB extracellular domain and human ActRIIA extracellular domain are illustrated in Figure 3. This alignment indicates amino acid residues within both receptors that are believed to directly contact ActRII ligands. For example, the composite ActRII structures indicated that the ActRIIB-ligand binding pocket is defined, in part, by residues Y31, N33, N35, L38 through T41, E47, E50, Q53 through K55, L57, H58, Y60, S62, K74, W78 through N83, Y85, R87, A92, and E94 through F101. At these positions, it is expected that conservative mutations will be tolerated.

In addition, ActRIIB is well-conserved among vertebrates, with large stretches of the extracellular domain completely conserved. For example, Figure 4 depicts a multi-sequence alignment of a human ActRIIB extracellular domain compared to various ActRIIB orthologs. Many of the ligands that bind to ActRIIB are also highly conserved. Accordingly, from these alignments, it is possible to predict key amino acid positions within the ligand-binding domain that are important for normal ActRIIB-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant of substitution without significantly altering normal ActRIIB-ligand binding activities. Therefore, an active, human ActRIIB variant polypeptide useful in accordance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ActRIIB, or may include a residue that is similar to that in the human or other vertebrate sequences. Without meaning to be limiting, the following examples illustrate this approach to defining an active ActRIIB variant. L46 in the human extracellular domain (SEQ ID NO: 2) is a valine in *Xenopus* ActRIIB (SEQ ID NO: 505), and so this position may be altered, and optionally may be altered to another hydrophobic residue, such as V, I or F, or a non-polar residue such as A. E52 in the human extracellular domain is a K in *Xenopus*, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y and probably A. T93 in the human extracellular domain is a K in *Xenopus*, indicating that a wide structural variation is tolerated at this position, with polar residues favored, such as S, K, R, E, D, H, G, P, G and Y. F108 in the human extracellular domain is a Y in *Xenopus*, and therefore Y or other hydrophobic group, such as I, V or L should be tolerated. E111 in the human extracellular domain is K in *Xenopus*, indicating that charged residues will be tolerated at this position, including D, R, K and H, as well as Q and N. R112 in the human extracellular domain is K in *Xenopus*, indicating that basic residues are tolerated at this position, including R and H. A at position 119 in the human extracellular domain is relatively poorly conserved, and appears as P in rodents and V in *Xenopus*, thus essentially any amino acid should be tolerated at this position.

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

generate ActRIIB variants that retain one or more normal activities (e.g., ligand-binding activity).

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

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

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

polypeptides ending at 128 (with respect to SEQ ID NO: 1) or later should retain ligand-binding activity. ActRIIB polypeptides ending at or between 119 and 127 (*e.g.*, 119, 120, 121, 122, 123, 124, 125, 126, or 127), with respect to SEQ ID NO: 1, will have an intermediate binding ability. Any of these forms may be desirable to use, depending on the clinical or experimental setting.

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

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

130, 131, 132, or 133) of SEQ ID NO: 1. Other examples include constructs that begin at a position from 20-24 (*e.g.*, any one of positions 20, 21, 22, 23, or 24), 21-24 (*e.g.*, any one of positions 21, 22, 23, or 24), or 22-25 (*e.g.*, any one of positions 22, 22, 23, or 25) of SEQ ID NO: 1 and end at a position from 109-134 (*e.g.*, any one of positions 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-134 (*e.g.*, any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) or 129-134 (*e.g.*, any one of positions 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Variants within these ranges are also contemplated, particularly those having at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 1.

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

position 81 (acidic), 98 (polar or charged, particularly E, D, R or K), all with respect to SEQ ID NO: 1.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ActRIIB polypeptides for use in accordance with the disclosure are soluble (*e.g.*, an extracellular domain of ActRIIB). In other preferred embodiments, ActRIIB polypeptides for use in accordance with the disclosure bind to one or more TGF-beta superfamily ligands. Therefore, in some embodiments, ActRIIB polypeptides for use in accordance with the disclosure inhibit (antagonize) activity (*e.g.*, inhibition of Smad signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists essentially of, or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to amino acids 20-29 (*e.g.*, beginning at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and ending at a position corresponding to amino acids 109-134 (*e.g.*, ending at any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. In certain preferred embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 29-109 of SEQ ID NO: 1 In other preferred embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 25-131 of SEQ ID NO: 1 In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In certain embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide wherein the amino acid position corresponding to L79 of SEQ ID NO: 1 is not an acidic amino acid (*i.e.*, is not a naturally occurring D or E amino acid residue or artificial acidic amino acid).

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

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

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  1 MGAAAKLAFA VFLISCSSGA ILGRSETQEC LFFNANWEKD RTNQTGVEPC
 51 YGDKDKRRHC FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKKDSPEV
101 YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPPYYNIL LYSLVPLMLI
151 AGIVICAFWV YRHHKMAYPP VLVPTQDPGP PPPSPLLGLK PLQLLEVKAR
201 GRFGCVWKAQ LLNEYVAVKI FPIQDKQSWQ NEYEVYSLPG MKHENILQFI
251 GAEKRGTSVD VDLWLITAFH EKGSLSDFLK ANVVSWNELC HIAETMARGL
301 AYLHEDIPGL KDGHKPAISH RDIKSKNVLL KNNLTACIAD FGLALKFEAG
351 KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR
401 CTAADGPVDE YMLPFEEEIG QHPSLEDMQE VVVHKKKRPV LRDYWQKHAG
451 MAMLCETIEE CWDHDAEARL SAGCVGERIT QMQRLTNIIT TEDIVTVVTM
501 VTNVDFPPKE SSL  (SEQ ID NO: 9)
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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 INCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPP (SEQ ID NO: 10)

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

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDD INCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEM (SEQ ID NO: 11)

A nucleic acid sequence encoding the human ActRIIA precursor protein is shown below (SEQ ID NO: 12), corresponding to nucleotides 159-1700 of Genbank Reference Sequence NM_001616.4. The signal sequence is underlined.

```
   1 ATGGGAGCTG CTGCAAAGTT GGCGTTTGCC GTCTTTCTTA TCTCCTGTTC
  51 TTCAGGTGCT ATACTTGGTA GATCAGAAAC TCAGGAGTGT CTTTTCTTTA
 101 ATGCTAATTG GGAAAAAGAC AGAACCAATC AAACTGGTGT TGAACCGTGT
 151 TATGGTGACA AAGATAAACG GCGGCATTGT TTTGCTACCT GGAAGAATAT
 201 TTCTGGTTCC ATTGAAATAG TGAAACAAGG TTGTTGGCTG GATGATATCA
 251 ACTGCTATGA CAGGACTGAT TGTGTAGAAA AAAAAGACAG CCCTGAAGTA
 301 TATTTTTGTT GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATTT
 351 TCCGGAGATG GAAGTCACAC AGCCCACTTC AAATCCAGTT ACACCTAAGC
 401 CACCCTATTA CAACATCCTG CTCTATTCCT TGGTGCCACT TATGTTAATT
 451 GCGGGGATTG TCATTTGTGC ATTTTGGGTG TACAGGCATC ACAAGATGGC
 501 CTACCCTCCT GTACTTGTTC CAACTCAAGA CCCAGGACCA CCCCCACCTT
 551 CTCCATTACT AGGTTTGAAA CCACTGCAGT TATTAGAAGT GAAAGCAAGG
 601 GGAAGATTTG GTTGTGTCTG GAAAGCCCAG TTGCTTAACG AATATGTGGC
 651 TGTCAAAATA TTTCCAATAC AGGACAAACA GTCATGGCAA AATGAATACG
 701 AAGTCTACAG TTTGCCTGGA ATGAAGCATG AGAACATATT ACAGTTCATT
 751 GGTGCAGAAA AACGAGGCAC CAGTGTTGAT GTGGATCTTT GGCTGATCAC
 801 AGCATTTCAT GAAAAGGGTT CACTATCAGA CTTTCTTAAG GCTAATGTGG
 851 TCTCTTGGAA TGAACTGTGT CATATTGCAG AAACCATGGC TAGAGGATTG
 901 GCATATTTAC ATGAGGATAT ACCTGGCCTA AAAGATGGCC ACAAACCTGC
 951 CATATCTCAC AGGGACATCA AAAGTAAAAA TGTGCTGTTG AAAAACAACC
1001 TGACAGCTTG CATTGCTGAC TTTGGGTTGG CCTTAAAATT TGAGGCTGGC
1051 AAGTCTGCAG GCGATACCCA TGGACAGGTT GGTACCCGGA GGTACATGGC
1101 TCCAGAGGTA TTAGAGGGTG CTATAAACTT CCAAAGGGAT GCATTTTTGA
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1151 GGATAGATAT GTATGCCATG GGATTAGTCC TATGGGAACT GGCTTCTCGC
1201 TGTACTGCTG CAGATGGACC TGTAGATGAA TACATGTTGC CATTTGAGGA
1251 GGAAATTGGC CAGCATCCAT CTCTTGAAGA CATGCAGGAA GTTGTTGTGC
1301 ATAAAAAAAA GAGGCCTGTT TTAAGAGATT ATTGGCAGAA ACATGCTGGA
1351 ATGGCAATGC TCTGTGAAAC CATTGAAGAA TGTTGGGATC ACGACGCAGA
1401 AGCCAGGTTA TCAGCTGGAT GTGTAGGTGA AAGAATTACC CAGATGCAGA
1451 GACTAACAAA TATTATTACC ACAGAGGACA TTGTAACAGT GGTCACAATG
1501 GTGACAAATG TTGACTTTCC TCCCAAAGAA TCTAGTCTA
```

(SEQ ID NO: 12)

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

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

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

In certain embodiments, the disclosure relates to 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.*, 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, 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, 118, 120, 409, or 410. In some embodiments, 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, 118, 120, 409, or 410.

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

The term "TGFBRII polypeptide" includes polypeptides comprising any naturally occurring polypeptide of a TGFBRII family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Numbering of amino acids for all TGFBRII-related polypeptides described herein is based on the numbering of the human TGFBRII precursor protein sequence below (SEQ ID NO: 42), unless specifically designated otherwise.

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

1 MGRGLLRGLW PLHIVLWTRI AS**TIPPHVQK SVNNDMIVTD NNGAVKFPQL**

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

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

The processed extracellular TGFBRII polypeptide sequence is as follows:

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVC
VAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDN
IIFSEEYNTSNPDLLLVIFQ (SEQ ID NO: 43)

The nucleic acid sequence encoding TGFBRII 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.

ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGC
CAGC**ACGATCCCACCGCACGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACA**
ACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGAC
AACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGT
CTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACC
CCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAG
GAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGA
CAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTC
AAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGTCATCATCATCTTC
TACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAACCGGCAAGACGCG
GAAGCTCATGGAGTTCAGCGAGCACTGTGCCATCATCCTGGAAGATGACCGCTCTGACATCA
GCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCCATTGAGCTGGACACC

CTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGCAGAACACTTCAGA
GCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCCTCTTGGAAGACAG
AGAAGGACATCTTCTCAGACATCAATCTGAAGCATGAGAACATACTCCAGTTCCTGACGGCT
GAGGAGCGGAAGACGGAGTTGGGGAAACAATACTGGCTGATCACCGCCTTCCACGCCAAGGG
CAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGACCTGCGCAAGCTGGGCA
GCTCCCTCGCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATGTGGGAGGCCCAAG
ATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGAACGACCTAACCTG
CTGCCTGTGTGACTTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCTGTGGATGACCTGG
CTAACAGTGGGCAGGTGGGAACTGCAAGATACATGGCTCCAGAAGTCCTAGAATCCAGGATG
AATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACTCCATGGCTCTGGTGCTCTG
GGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAGCCTCCATTTGGTT
CCAAGGTGCGGGAGCACCCCTGTGTCGAAAGCATGAAGGACAACGTGTTGAGAGATCGAGGG
CGACCAGAAATTCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGGTGTGTGAGACGTT
GACTGAGTGCTGGGACCACGACCCAGAGGCCCGTCTCACAGCCCAGTGTGTGGCAGAACGCT
TCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGCTGCTCGGAGGAGAAGATTCCT
GAAGACGGCTCCCTAAACACTACCAAA (SEQ ID NO: 44)

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

ACGATCCCACCGCACGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACAACGG
TGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACC
AGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGTCTGT
GTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACCCCAA
GCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAA
AAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGACAAC
ATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAA
(SEQ ID NO: 45)

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

1 MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT
51 AHPLRHINND MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS
101 ICEKPQEVCV AVWRKNDENI TLETVCHDPK LPYHDFILED AASPKCIMKE
151 KKKPGETFFM CSCSSDECND NIIFSEEYNT SNPDLLLVIF QVTGISLLPP
201 LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH CAIILEDDRS

251 DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA
301 VKIFPYEEYA SWKTEKDIFS DINLKHENIL QFLTAEERKT ELGKQYWLIT
351 AFHAKGNLQE YLTRHVISWE DLRKLGSSLA RGIAHLHSDH TPCGRPKMPI
401 VHRDLKSSNI LVKNDLTCCL CDFGLSLRLD PTLSVDDLAN SGQVGTARYM
451 APEVLESRMN LENVESFKQT DVYSMALVLW EMTSRCNAVG EVKDYEPPFG
501 SKVREHPCVE SMKDNVLRDR GRPEIPSFWL NHQGIQMVCE TLTECWDHDP
551 EARLTAQCVA ERFSELEHLD RLSGRSCSEE KIPEDGSLNT TK
(SEQ ID NO: 67)

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

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

TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFS
TCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKC
IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQ (SEQ ID NO: 68)

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

ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGC
CAGC**ACGATCCCACCGCACGTTCAGAAGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAA**
TCATCTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATA
GTCACTGACAACAACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATT
TTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGA
AGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACA
GTTTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAA
GTGCATTATGAAGGAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTG
ATGAGTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTG
CTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGT
CATCATCATCTTCTACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAA
CCGGCAAGACGCGGAAGCTCATGGAGTTCAGCGAGCACTGTGCCATCATCCTGGAAGATGAC
CGCTCTGACATCAGCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCCAT
TGAGCTGGACACCCTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGC
AGAACACTTCAGAGCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCC

TCTTGGAAGACAGAGAAGGACATCTTCTCAGACATCAATCTGAAGCATGAGAACATACTCCA
GTTCCTGACGGCTGAGGAGCGGAAGACGGAGTTGGGGAAACAATACTGGCTGATCACCGCCT
TCCACGCCAAGGGCAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGACCTG
CGCAAGCTGGGCAGCTCCCTCGCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATG
TGGGAGGCCCAAGATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGA
ACGACCTAACCTGCTGCCTGTGTGACTTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCT
GTGGATGACCTGGCTAACAGTGGGCAGGTGGGAACTGCAAGATACATGGCTCCAGAAGTCCT
AGAATCCAGGATGAATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACTCCATGG
CTCTGGTGCTCTGGGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAG
CCTCCATTTGGTTCCAAGGTGCGGGAGCACCCCTGTGTCGAAAGCATGAAGGACAACGTGTT
GAGAGATCGAGGGCGACCAGAAATTCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGG
TGTGTGAGACGTTGACTGAGTGCTGGGACCACGACCCAGAGGCCCGTCTCACAGCCCAGTGT
GTGGCAGAACGCTTCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGCTGCTCGGA
GGAGAAGATTCCTGAAGACGGCTCCCTAAACACTACCAAA (SEQ ID NO: 69)

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

ACGATCCCACCGCACGTTCAGAAGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAATCAT
CTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATAGTCA
CTGACAACAACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCC
ACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCC
ACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTT
GCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGC
ATTATGAAGGAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGA
GTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAG
TCATATTTCAA (SEQ ID NO: 70).

Either of the foregoing TGFβRII isoforms (SEQ ID NOs: 42, 43, 67, and 68) could incorporate an insertion of 36 amino acids (SEQ ID NO: 95) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 42; positions 129 and 130 of SEQ ID NO: 43; positions 176 and 177 of SEQ ID NO: 67; or positions 154 and 155 of SEQ ID NO: 68) located near the C-terminus of the TGFβRII ECD, as occurs naturally in the TGFβRII isoform C (Konrad et al., BMC Genomics 8:318, 2007).

GRCKIRHIGS NNRLQRSTCQ NTGWESAHVM KTPGFR (SEQ ID NO: 95)

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

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

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

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

(SEQ ID NO: 46)

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

The processed extracellular BMPRII polypeptide sequence is as follows:

SQNQERLCAFKDPYQQDLGIGESRISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHI
GDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET
(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
CAGCACTGCGGCTGCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGC
AAGACCTTGGGATAGGTGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAA
GGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGG
ATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCA
CTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAAT
GTCAACTTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATT
TAACCGAGATGAGACAATAATCATTGCTTTGGCATCAGTCTCTGTATTAGCTGTTTTGATAG
TTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAAACAAGGTCTTCACAGTATG
AACATGATGGAGGCAGCAGCATCCGAACCCTCTCTTGATCTAGATAATCTGAAACTGTTGGA
GCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTG
CTGTAAAAGTGTTTTCCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGA
GTGCCTTTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGC
AGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATCCCAATGGATCTTTATGCAAGT
ATTTAAGTCTCCACACAAGTGACTGGGTAAGCTCTTGCCGTCTTGCTCATTCTGTTACTAGA
GGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCA
TCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACT
TTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCA
GCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAA
CTTGAGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGACTAATCTATT
GGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATG
GCTTTTCAGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAGGTTCTCGTGTCTAG
GGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCAC
TCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGT
GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGCAACCTGTCACATAATAGGCGTGTGC
CAAAAATTGGTCCTTATCCAGATTATTCTTCCTCCTCATACATTGAAGACTCTATCCATCAT
ACTGACAGCATCGTGAAGAATATTTCCTCTGAGCATTCTATGTCCAGCACACCTTTGACTAT
AGGGGAAAAAAACCGAAATTCAATTAACTATGAACGACAGCAAGCACAAGCTCGAATCCCCA
GCCCTGAAACAAGTGTCACCAGCCTCTCCACCAACACAACAACCACAAACACCACAGGACTC
ACGCCAAGTACTGGCATGACTACTATATCTGAGATGCCATACCCAGATGAAACAAATCTGCA
TACCACAAATGTTGCACAGTCAATTGGGCCAACCCCTGTCTGCTTACAGCTGACAGAAGAAG
ACTTGGAAACCAACAAGCTAGACCCAAAAGAAGTTGATAAGAACCTCAAGGAAAGCTCTGAT
GAGAATCTCATGGAGCACTCTCTTAAACAGTTCAGTGGCCCAGACCCACTGAGCAGTACTAG

TTCTAGCTTGCTTTACCCACTCATAAAACTTGCAGTAGAAGCAACTGGACAGCAGGACTTCA
CACAGACTGCAAATGGCCAAGCATGTTTGATTCCTGATGTTCTGCCTACTCAGATCTATCCT
CTCCCCAAGCAGCAGAACCTTCCCAAGAGACCTACTAGTTTGCCTTTGAACACCAAAAATTC
AACAAAAGAGCCCCGGCTAAAATTTGGCAGCAAGCACAAATCAAACTTGAAACAAGTCGAAA
CTGGAGTTGCCAAGATGAATACAATCAATGCAGCAGAACCTCATGTGGTGACAGTCACCATG
AATGGTGTGGCAGGTAGAAACCACAGTGTTAACTCCCATGCTGCCACAACCCAATATGCCAA
TGGGACAGTACTATCTGGCCAAACAACCAACATAGTGACACATAGGGCCCAAGAAATGTTGC
AGAATCAGTTTATTGGTGAGGACACCCGGCTGAATATTAATTCCAGTCCTGATGAGCATGAG
CCTTTACTGAGACGAGAGCAACAAGCTGGCCATGATGAAGGTGTTCTGGATCGTCTTGTGGA
CAGGAGGGAACGGCCACTAGAAGGTGGCCGAACTAATTCCAATAACAACAACAGCAATCCAT
GTTCAGAACAAGATGTTCTTGCACAGGGTGTTCCAAGCACAGCAGCAGATCCTGGGCCATCA
AAGCCCAGAAGAGCACAGAGGCCTAATTCTCTGGATCTTTCAGCCACAAATGTCCTGGATGG
CAGCAGTATACAGATAGGTGAGTCAACACAAGATGGCAAATCAGGATCAGGTGAAAAGATCA
AGAAACGTGTGAAAACTCCCTATTCTCTTAAGCGGTGGCGCCCCTCCACCTGGGTCATCTCC
ACTGAATCGCTGGACTGTGAAGTCAACAATAATGGCAGTAACAGGGCAGTTCATTCCAAATC
CAGCACTGCTGTTTACCTTGCAGAAGGAGGCACTGCTACAACCATGGTGTCTAAAGATATAG
GAATGAACTGTCTG (SEQ ID NO: 48)

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

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGG
TGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATG
GCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGGATGTTGGTCTCACATT
GGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCACTCCTCCCTCAATTCA
GAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGAGA
ATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
(SEQ ID NO: 49)

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

1 MTSSLQRPWR VPWLPWTILL VSTAAA**SQNQ ERLCAFKDPY QQDLGIGESR**
51 ISHENGTILC SKGSTCYGLW EKSKGDINLV KQGCWSHIGD PQECHYEECV
101 VTTTPPSIQN GTYRFCCCST DLCNVNFTEN FPPPDTTPLS PPHSFNRDET
151 IIIALASVSV LAVLIVALCF GYRMLTGDRK QGLHSMNMME AAASEPSLDL
201 DNLKLLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNF INEKNIYRVP

251 LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS
301 SCRLAHSVTR GLAYLHTELP RGDHYKPAIS HRDLNSRNVL VKNDGTCVIS
351 DFGLSMRLTG NRLVRPGEED NAAISEVGTI RYMAPEVLEG AVNLRDCESA
401 LKQVDMYALG LIYWEIFMRC TDLFPGESVP EYQMAFQTEV GNHPTFEDMQ
451 VLVSREKQRP KFPEAWKENS LAVRSLKETI EDCWDQDAEA RLTAQCAEER
501 MAELMMIWER NKSVSPTVNP MSTAMQNERR (SEQ ID NO: 71)

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:

SQNQERLCAFKDPYQQDLGIGESRISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHI
GDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET
(SEQ ID NO: 72)

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
AAGACCTTGGGATAGGTGAGAGTAGAATCTCTCATGAAAATGGGACAATATTATGCTCGAAA
GGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGG
ATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCA
CTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAAT
GTCAACTTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATT
TAACCGAGATGAGACAATAATCATTGCTTTGGCATCAGTCTCTGTATTAGCTGTTTTGATAG
TTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAAACAAGGTCTTCACAGTATG
AACATGATGGAGGCAGCAGCATCCGAACCCTCTCTTGATCTAGATAATCTGAAACTGTTGGA
GCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTG
CTGTAAAAGTGTTTTCCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGA
GTGCCTTTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGC
AGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATCCCAATGGATCTTTATGCAAGT
ATTTAAGTCTCCACACAAGTGACTGGGTAAGCTCTTGCCGTCTTGCTCATTCTGTTACTAGA
GGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCA

TCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACT
TTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCA
GCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAA
CTTGAGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGACTAATCTATT
GGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATG
GCTTTTCAGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAGGTTCTCGTGTCTAG
GGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCAC
TCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGT
GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGTAGG (SEQ ID NO: 73)

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

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

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

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

The term "MISRII polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an MISRII family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. 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 NP_065434.1) is as follows:

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

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

The processed extracellular MISRII polypeptide sequence is as follows:

PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
RDSDEPGCESLHCDPSPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
GESIWMAL (SEQ ID NO: 51)

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

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCA**CCCCCAAACAG**
GCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGC
TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGA
TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGGCT
CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
CTGGATGGCACTGGTGCTGCTGGGGCTGTTCCTCCTCCTCCTGCTGCTGCTGGGCAGCATCA
TCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGAGCCAGTGCCAGAGCCAAGG
CCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCA
GGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACTGGTTG
CCATCAAGGCCTTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTGTACGAA
CTTCCAGGCCTACAGCACGACCACATTGTCCGATTTATCACTGCCAGCCGGGGGGGTCCTGG
CCGCCTGCTCTCTGGGCCCCTGCTGGTACTGGAACTGCATCCCAAGGGCTCCCTGTGCCACT
ACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTGGCCCAG
GGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCA
CCGAGATCTGAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACC
TGGGCCTTGCCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCA
CAAGGCCCAGCTGCCATCATGGAAGCTGGCACCCAGAGGTACATGGCACCAGAGCTCTTGGA
CAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTACTCTTTGG
CTCTGCTCCTGTGGGAGATACTGAGCCGCTGCCCAGATTTGAGGCCTGACAGCAGTCCACCA
CCCTTCCAACTGGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGC
CTTGGCAGTGCAGGAGAGGAGGCGTCCCTACATCCCATCCACCTGGCGCTGCTTTGCCACAG
ACCCTGATGGGCTGAGGGAGCTCCTAGAAGACTGTTGGGATGCAGACCCAGAAGCACGGCTG
ACAGCTGAGTGTGTACAGCAGCGCCTGGCTGCCTTGGCCCATCCTCAAGAGAGCCACCCCTT
TCCAGAGAGCTGTCCACGTGGCTGCCCACCTCTCTGCCCAGAAGACTGTACTTCAATTCCTG

CCCCTACCATCCTCCCCTGTAGGCCTCAGCGGAGTGCCTGCCACTTCAGCGTTCAGCAAGGC
CCTTGTTCCAGGAATCCTCAGCCTGCCTGTACCCTTTCTCCTGTG (SEQ ID NO: 52)

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

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

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

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

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

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

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

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
GCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGC
TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGA
TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGGCT
CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
CTGGATGGCACTGGTGCTGCTGGGGCTGTTCCTCCTCCTCCTGCTGCTGCTGGGCAGCATCA
TCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGAGCCAGTGCCAGAGCCAAGG
CCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCA
GGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACTGGTTG
CCATCAAGGCCTTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTGTACGAA
CTTCCAGGCCTACAGCACGACCACATTGTCCGATTTATCACTGCCAGCCGGGGGGGTCCTGG
CCGCCTGCTCTCTGGGCCCCTGCTGGTACTGGAACTGCATCCCAAGGGCTCCCTGTGCCACT
ACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTGGCCCAG
GGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCA
CCGAGATCTGAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACC
TGGGCCTTGCCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCA
CAAGGCCCAGCTGCCATCATGGAAGCTGGCACCCAGAGGTACATGGCACCAGAGCTCTTGGA
CAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTACTCTTTGG
CTCTGCTCCTGTGGGAGATACTGAGCCGCTGCCCAGATTTGAGGCCTGCAGTCCACCACCCT
TCCAACTGGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGCCTTG
GCAGTGCAGGAGAGGAGGCGTCCCTACATCCCATCCACCTGGCGCTGCTTTGCCACAGACCC
TGATGGGC (SEQ ID NO: 77)

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

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

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

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

A nucleic acid sequence encoding human MISRII precursor protein (isoform 3) is shown below (SEQ ID NO: 81), corresponding to nucleotides 81-1514 of Genbank Reference Sequence NM 001164691.1. The signal sequence is underlined.

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAG
GCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGC
TGCTAGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTT
GGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGA
TGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGGCT
CCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCT
CCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCAT
CTGGATGGCACTGGTGCTGCTGGGGCTGTTCCTCCTCCTCCTGCTGCTGCTGGGCAGCATCA
TCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGAGCCAGTGCCAGAGCCAAGG
CCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCTCCCA
GGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACTGGTTG
CCATCAAGGCCTTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTGTACGAA

CTTCCAGGCCTACAGCACGACCACATTGTCCGATTTATCACTGCCAGCCGGGGGGGTCCTGG
CCGCCTGCTCTCTGGGCCCCTGCTGGTACTGGAACTGCATCCCAAGGGCTCCCTGTGCCACT
ACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTGGCCCAG
GGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCA
CCGAGATCTGAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACC
TGGGCCTTGCCTTGGTGCTCCCTGGCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCA
CAAGGCCCAGCTGCCATCATGGAAGACCCTGATGGGCTGAGGGAGCTCCTAGAAGACTGTTG
GGATGCAGACCCAGAAGCACGGCTGACAGCTGAGTGTGTACAGCAGCGCCTGGCTGCCTTGG
CCCATCCTCAAGAGAGCCACCCCTTTCCAGAGAGCTGTCCACGTGGCTGCCCACCTCTCTGC
CCAGAAGACTGTACTTCAATTCCTGCCCCTACCATCCTCCCCTGTAGGCCTCAGCGGAGTGC
CTGCCACTTCAGCGTTCAGCAAGGCCCTTGTTCCAGGAATCCTCAGCCTGCCTGTACCCTTT
CTCCTGTG (SEQ ID NO: 81)

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

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

In certain embodiments, the disclosure relates to 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.*, heteromultimer complexes comprising a MISRII polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of MISRII). In other preferred embodiments, MISRII polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, 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, or 80. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one MISRII polypeptide

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

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

The term "ALK1 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK1 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. 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 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 RHPQEHRGCG NLHRELCRGR PTEFVNHYCC DSHLCNHNVS
101 LVLEATQPPS EQPGTDGQLA LILGPVLALL ALVALGVLGL WHVRRRQEKQ
151 RGLHSELGES SLILKASEQG DSMLGDLLDS DCTTGSGSGL PFLVQRTVAR
201 QVALVECVGK GRYGEVWRGL WHGESVAVKI FSSRDEQSWF RETEIYNTVL
251 LRHDNILGFI ASDMTSRNSS TQLWLITHYH EHGSLYDFLQ RQTLEPHLAL
301 RLAVSAACGL AHLHVEIFGT QGKPAIAHRD FKSRNVLVKS NLQCCIADLG
351 LAVMHSQGSD YLDIGNNPRV GTKRYMAPEV LDEQIRTDCF ESYKWTDIWA
401 FGLVLWEIAR RTIVNGIVED YRPPFYDVVP NDPSFEDMKK VVCVDQQTPT
451 IPNRLAADPV LSGLAQMMRE CWYPNPSARL TALRIKKTLQ KISNSPEKPK
501 VIQ (SEQ ID NO: 14)

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

The processed extracelluar ALK1 polypeptide sequence is as follows:

DPVKPSRGPLVTCTCESPHCKGPTCRGAWCTVVLVREEGRHPQEHRGCGNLHRELCRGRPTE
FVNHYCCDSHLCNHNVSLVLEATQPPSEQPGTDGQ (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.

ATGACCTTGGGCTCCCCCAGGAAAGGCCTTCTGATGCTGCTGATGGCCTTGGTGACCCAGGG
A**GACCCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACCTGCACGTGTGAGAGCCCACATTGCA**
AGGGGCCTACCTGCCGGGGGGCCTGGTGCACAGTAGTGCTGGTGCGGGAGGAGGGGAGGCAC
CCCCAGGAACATCGGGGCTGCGGGAACTTGCACAGGGAGCTCTGCAGGGGGCGCCCCACCGA
GTTCGTCAACCACTACTGCTGCGACAGCCACCTCTGCAACCACAACGTGTCCCTGGTGCTGG
AGGCCACCCAACCTCCTTCGGAGCAGCCGGGAACAGATGGCCAGCTGGCCCTGATCCTGGGC
CCCGTGCTGGCCTTGCTGGCCCTGGTGGCCCTGGGTGTCCTGGGCCTGTGGCATGTCCGACG
GAGGCAGGAGAAGCAGCGTGGCCTGCACAGCGAGCTGGGAGAGTCCAGTCTCATCCTGAAAG
CATCTGAGCAGGGCGACAGCATGTTGGGGGACCTCCTGGACAGTGACTGCACCACAGGGAGT
GGCTCAGGGCTCCCCTTCCTGGTGCAGAGGACAGTGGCACGGCAGGTTGCCTTGGTGGAGTG
TGTGGGAAAAGGCCGCTATGGCGAAGTGTGGCGGGGCTTGTGGCACGGTGAGAGTGTGGCCG
TCAAGATCTTCTCCTCGAGGGATGAACAGTCCTGGTTCCGGGAGACTGAGATCTATAACACA
GTGTTGCTCAGACACGACAACATCCTAGGCTTCATCGCCTCAGACATGACCTCCCGCAACTC
GAGCACGCAGCTGTGGCTCATCACGCACTACCACGAGCACGGCTCCCTCTACGACTTTCTGC
AGAGACAGACGCTGGAGCCCCATCTGGCTCTGAGGCTAGCTGTGTCCGCGGCATGCGGCCTG
GCGCACCTGCACGTGGAGATCTTCGGTACACAGGGCAAACCAGCCATTGCCCACCGCGACTT
CAAGAGCCGCAATGTGCTGGTCAAGAGCAACCTGCAGTGTTGCATCGCCGACCTGGGCCTGG
CTGTGATGCACTCACAGGGCAGCGATTACCTGGACATCGGCAACAACCCGAGAGTGGGCACC
AAGCGGTACATGGCACCCGAGGTGCTGGACGAGCAGATCCGCACGGACTGCTTTGAGTCCTA
CAAGTGGACTGACATCTGGGCCTTTGGCCTGGTGCTGTGGGAGATTGCCCGCCGGACCATCG
TGAATGGCATCGTGGAGGACTATAGACCACCCTTCTATGATGTGGTGCCCAATGACCCCAGC
TTTGAGGACATGAAGAAGGTGGTGTGTGTGGATCAGCAGACCCCCACCATCCCTAACCGGCT
GGCTGCAGACCCGGTCCTCTCAGGCCTAGCTCAGATGATGCGGGAGTGCTGGTACCCAAACC
CCTCTGCCCGACTCACCGCGCTGCGGATCAAGAAGACACTACAAAAAATTAGCAACAGTCCA
GAGAAGCCTAAAGTGATTCAA (SEQ ID NO: 16)

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

GACCCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACCTGCACGTGTGAGAGCCCACATTGCAA
GGGGCCTACCTGCCGGGGGGCCTGGTGCACAGTAGTGCTGGTGCGGGAGGAGGGGAGGCACC
CCCAGGAACATCGGGGCTGCGGGAACTTGCACAGGGAGCTCTGCAGGGGGCGCCCCACCGAG
TTCGTCAACCACTACTGCTGCGACAGCCACCTCTGCAACCACAACGTGTCCCTGGTGCTGGA
GGCCACCCAACCTCCTTCGGAGCAGCCGGGAACAGATGGCCAG (SEQ ID NO: 17)

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

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

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

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

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

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

The processed extracellular ALK2 polypeptide sequence is as follows:

MEDEKPKVNPKLYMCVCEGLSCGNEDHCEGQQCFSSLSINDGFHVYQKGCFQVYEQGKMTCK
TPPSPGQAVECCQGDWCNRNITAQLPTKGKSFPGTQNFHLE (SEQ ID NO: 19)

A nucleic acid sequence encoding human ALK2 precursor protein is shown 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
CCCGCCGTCCCCTGGCCAAGCCGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACATCA
CGGCCCAGCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAGGTT
GGCCTCATTATTCTCTCTGTAGTGTTCGCAGTATGTCTTTTAGCCTGCCTGCTGGGAGTTGC
TCTCCGAAAATTTAAAAGGCGCAACCAAGAACGCCTCAATCCCCGAGACGTGGAGTATGGCA
CTATCGAAGGGCTCATCACCACCAATGTTGGAGACAGCACTTTAGCAGATTTATTGGATCAT
TCGTGTACATCAGGAAGTGGCTCTGGTCTTCCTTTTCTGGTACAAAGAACAGTGGCTCGCCA
GATTACACTGTTGGAGTGTGTCGGGAAAGGCAGGTATGGTGAGGTGTGGAGGGGCAGCTGGC

AAGGGGAGAATGTTGCCGTGAAGATCTTCTCCTCCCGTGATGAGAAGTCATGGTTCAGGGAA
ACGGAATTGTACAACACTGTGATGCTGAGGCATGAAAATATCTTAGGTTTCATTGCTTCAGA
CATGACATCAAGACACTCCAGTACCCAGCTGTGGTTAATTACACATTATCATGAAATGGGAT
CGTTGTACGACTATCTTCAGCTTACTACTCTGGATACAGTTAGCTGCCTTCGAATAGTGCTG
TCCATAGCTAGTGGTCTTGCACATTTGCACATAGAGATATTTGGGACCCAAGGGAAACCAGC
CATTGCCCATCGAGATTTAAAGAGCAAAAATATTCTGGTTAAGAAGAATGGACAGTGTTGCA
TAGCAGATTTGGGCCTGGCAGTCATGCATTCCCAGAGCACCAATCAGCTTGATGTGGGGAAC
AATCCCCGTGTGGGCACCAAGCGCTACATGGCCCCCGAAGTTCTAGATGAAACCATCCAGGT
GGATTGTTTCGATTCTTATAAAAGGGTCGATATTTGGGCCTTTGGACTTGTTTTGTGGGAAG
TGGCCAGGCGGATGGTGAGCAATGGTATAGTGGAGGATTACAAGCCACCGTTCTACGATGTG
GTTCCCAATGACCCAAGTTTTGAAGATATGAGGAAGGTAGTCTGTGTGGATCAACAAAGGCC
AAACATACCCAACAGATGGTTCTCAGACCCGACATTAACCTCTCTGGCCAAGCTAATGAAAG
AATGCTGGTATCAAAATCCATCCGCAAGACTCACAGCACTGCGTATCAAAAAGACTTTGACC
AAAATTGATAATTCCCTCGACAAATTGAAAACTGACTGT (SEQ ID NO: 20)

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

ATGGAAGATGAGAAGCCCAAGGTCAACCCCAAACTCTACATGTGTGTGTGTGAAGGTCTCTC
CTGCGGTAATGAGGACCACTGTGAAGGCCAGCAGTGCTTTTCCTCACTGAGCATCAACGATG
GCTTCCACGTCTACCAGAAAGGCTGCTTCCAGGTTTATGAGCAGGGAAAGATGACCTGTAAG
ACCCCGCCGTCCCCTGGCCAAGCCGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACAT
CACGGCCCAGCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAG
(SEQ ID NO: 21)

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

disclosure consist or consist essentially of at least one ALK2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 18 or 19.

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

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

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

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

The processed extracellular ALK3 polypeptide sequence is as follows:

```
1 QNLDSMLHGT GMKSDSDQKK SENGVTLAPE DTLPFLKCYC SGHCPDDAIN NTCITNGHCF
```

61 AIIEEDDQGE TTLASGCMKY EGSDFQCKDS PKAQLRRTIE CCRTNLCNQY LQPTLPPVVI
121 GPFFDGSIR (SEQ ID NO: 23)

A nucleic acid sequence encoding human ALK3 precursor protein is shown 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 ATTTGTTCAT
CATTTCTCGT
61 GTTCAAGGAC **AGAATCTGGA TAGTATGCTT CATGGCACTG GGATGAAATC**
AGACTCCGAC
121 CAGAAAAAGT CAGAAAATGG AGTAACCTTA GCACCAGAGG ATACCTTGCC
TTTTTTAAAG
181 TGCTATTGCT CAGGGCACTG TCCAGATGAT GCTATTAATA ACACATGCAT
AACTAATGGA
241 CATTGCTTTG CCATCATAGA AGAAGATGAC CAGGGAGAAA CCACATTAGC
TTCAGGGTGT
301 ATGAAATATG AAGGATCTGA TTTTCAGTGC AAAGATTCTC CAAAAGCCCA
GCTACGCCGG
361 ACAATAGAAT GTTGTCGGAC CAATTTATGT AACCAGTATT TGCAACCCAC
ACTGCCCCCT
421 GTTGTCATAG GTCCGTTTTT TGATGGCAGC ATTCGATGGC TGGTTTTGCT
CATTTCTATG
481 GCTGTCTGCA TAATTGCTAT GATCATCTTC TCCAGCTGCT TTTGTTACAA
ACATTATTGC
541 AAGAGCATCT CAAGCAGACG TCGTTACAAT CGTGATTTGG AACAGGATGA
AGCATTTATT
601 CCAGTTGGAG AATCACTAAA AGACCTTATT GACCAGTCAC AAAGTTCTGG
TAGTGGGTCT
661 GGACTACCTT TATTGGTTCA GCGAACTATT GCCAAACAGA TTCAGATGGT
CCGGCAAGTT
721 GGTAAAGGCC GATATGGAGA AGTATGGATG GGCAAATGGC GTGGCGAAAA
AGTGGCGCTC
781 AAAGTATTCT TTACCACTGA AGAAGCCAGC TGGTTTCGAG AAACAGAAAT
CTACCAAACT
841 GTGCTAATGC GCCATGAAAA CATACTTGGT TTCATAGCGG CAGACATTAA
AGGTACAGGT

901 TCCTGGACTC AGCTCTATTT GATTACTGAT TACCATGAAA ATGGATCTCT
CTATGACTTC
961 CTGAAATGTG CTACACTGGA CACCAGAGCC CTGCTTAAAT TGGCTTATTC
AGCTGCCTGT
1021 GGTCTGTGCC ACCTGCACAC AGAAATTTAT GGCACCCAAG GAAAGCCCGC
AATTGCTCAT
1081 CGAGACCTAA AGAGCAAAAA CATCCTCATC AAGAAAAATG GGAGTTGCTG
CATTGCTGAC
1141 CTGGGCCTTG CTGTTAAATT CAACAGTGAC ACAAATGAAG TTGATGTGCC
CTTGAATACC
1201 AGGGTGGGCA CCAAACGCTA CATGGCTCCC GAAGTGCTGG ACGAAAGCCT
GAACAAAAAC
1261 CACTTCCAGC CCTACATCAT GGCTGACATC TACAGCTTCG GCCTAATCAT
TTGGGAGATG
1321 GCTCGTCGTT GTATCACAGG AGGGATCGTG GAAGAATACC AATTGCCATA
TTACAACATG
1381 GTACCGAGTG ATCCGTCATA CGAAGATATG CGTGAGGTTG TGTGTGTCAA
ACGTTTGCGG
1441 CCAATTGTGT CTAATCGGTG GAACAGTGAT GAATGTCTAC GAGCAGTTTT
GAAGCTAATG
1501 TCAGAATGCT GGGCCCACAA TCCAGCCTCC AGACTCACAG CATTGAGAAT
TAAGAAGACG
1561 CTTGCCAAGA TGGTTGAATC CCAAGATGTA AAAATC (SEQ ID NO: 24)

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

1 CAGAATCTGG ATAGTATGCT TCATGGCACT GGGATGAAAT CAGACTCCGA
CCAGAAAAAG
61 TCAGAAAATG GAGTAACCTT AGCACCAGAG GATACCTTGC CTTTTTTAAA
GTGCTATTGC
121 TCAGGGCACT GTCCAGATGA TGCTATTAAT AACACATGCA TAACTAATGG
ACATTGCTTT
181 GCCATCATAG AAGAAGATGA CCAGGGAGAA ACCACATTAG CTTCAGGGTG
TATGAAATAT
241 GAAGGATCTG ATTTTCAGTG CAAAGATTCT CCAAAAGCCC AGCTACGCCG
GACAATAGAA

301 TGTTGTCGGA CCAATTTATG TAACCAGTAT TTGCAACCCA CACTGCCCCC
TGTTGTCATA
361 GGTCCGTTTT TTGATGGCAG CATTCGA (SEQ ID NO: 25)

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

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

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

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

The term "ALK4 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK4 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. 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 canonical human ALK4 precursor protein sequence (NCBI Ref Seq NP_004293) is as follows:

1 MAESAGASSF FPLVVLLLAG SGG**SGPRGVQ ALLCACTSCL QANYTCETDG ACMVSIFNLD**

61 **GMEHHVRTCI PKVELVPAGK PFYCLSSEDL RNTHCCYTDY CNRIDLRVPS GHLKEPEHPS**

121 **MWGPVE**LVGI IAGPVFLLFL IIIIVFLVIN YHQRVYHNRQ RLDMEDPSCE MCLSKDKTLQ

181 DLVYDLSTSG SGSGLPLFVQ RTVARTIVLQ EIIGKGRFGE VWRGRWRGGD VAVKIFSSRE

241 ERSWFREAEI YQTVMLRHEN ILGFIAADNK DNGTWTQLWL VSDYHEHGSL FDYLNRYTVT

301 IEGMIKLALS AASGLAHLHM EIVGTQGKPG IAHRDLKSKN ILVKKNGMCA IADLGLAVRH

361 DAVTDTIDIA PNQRVGTKRY MAPEVLDETI NMKHFDSFKC ADIYALGLVY WEIARRCNSG

421 GVHEEYQLPY YDLVPSDPSI EEMRKVVCDQ KLRPNIPNWW QSYEALRVMG KMMRECWYAN

481 GAARLTALRI KKTLSQLSVQ EDVKI (SEQ ID NO: 26)

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

The processed extracellular human ALK4 polypeptide sequence is as follows:

SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGKPFYCL 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.

ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGCCGGCAG
CGGCGGG**TCCGGGCCCCGGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGG**
CCAACTACACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATG
GAGCACCATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTA
CTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGA
TCGACTTGAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCG
GTGGAGCTGGTAGGCATCATCGCCGGCCCGGTGTTCCTCCTGTTCCTCATCATCATCATTGT
TTTCCTTGTCATTAACTATCATCAGCGTGTCTATCACAACCGCCAGAGACTGGACATGGAAG
ATCCCTCATGTGAGATGTGTCTCTCCAAAGACAAGACGCTCCAGGATCTTGTCTACGATCTC
TCCACCTCAGGGTCTGGCTCAGGGTTACCCCTCTTTGTCCAGCGCACAGTGGCCCGAACCAT
CGTTTTACAAGAGATTATTGGCAAGGGTCGGTTTGGGGAAGTATGGCGGGGCCGCTGGAGGG
GTGGTGATGTGGCTGTGAAAATATTCTCTTCTCGTGAAGAACGGTCTTGGTTCAGGGAAGCA
GAGATATACCAGACGGTCATGCTGCGCCATGAAAACATCCTTGGATTTATTGCTGCTGACAA
TAAAGATAATGGCACCTGGACACAGCTGTGGCTTGTTTCTGACTATCATGAGCACGGGTCCC
TGTTTGATTATCTGAACCGGTACACAGTGACAATTGAGGGGATGATTAAGCTGGCCTTGTCT
GCTGCTAGTGGGCTGGCACACCTGCACATGGAGATCGTGGGCACCCAAGGGAAGCCTGGAAT
TGCTCATCGAGACTTAAAGTCAAAGAACATTCTGGTGAAGAAAAATGGCATGTGTGCCATAG
CAGACCTGGGCCTGGCTGTCCGTCATGATGCAGTCACTGACACCATTGACATTGCCCCGAAT
CAGAGGGTGGGGACCAAACGATACATGGCCCCTGAAGTACTTGATGAAACCATTAATATGAA
ACACTTTGACTCCTTTAAATGTGCTGATATTTATGCCCTCGGGCTTGTATATTGGGAGATTG
CTCGAAGATGCAATTCTGGAGGAGTCCATGAAGAATATCAGCTGCCATATTACGACTTAGTG
CCCTCTGACCCTTCCATTGAGGAAATGCGAAAGGTTGTATGTGATCAGAAGCTGCGTCCCAA
CATCCCCAACTGGTGGCAGAGTTATGAGGCACTGCGGGTGATGGGGAAGATGATGCGAGAGT
GTTGGTATGCCAACGGCGCAGCCCGCCTGACGGCCCTGCGCATCAAGAAGACCCTCTCCCAG
CTCAGCGTGCAGGAAGACGTGAAGATC (SEQ ID NO: 28)

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

TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTA
CACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACC
ATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTACTGCCTG

AGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGATCGACTT GAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAG (SEQ ID NO: 29)

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

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

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

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

SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGKPFYCL SSEDLRNTHCCYTDYCNRIDLRVPSGHLKEPEHPSMWGPVE (SEQ ID NO: 84)

A nucleic acid sequence encoding the ALK4 precursor protein (isoform C) is shown below (SEQ ID NO: 85), corresponding to nucleotides 78-1715 of Genbank Reference Sequence NM_020328.3. The signal sequence is underlined and the extracellular domain is indicated in **bold** font.

ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGCCGGCAG
CGGCGGG**TCCGGGCCCCGGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGG**
CCAACTACACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATG
GAGCACCATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTA
CTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGA
TCGACTTGAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCG
GTGGAGCTGGTAGGCATCATCGCCGGCCCGGTGTTCCTCCTGTTCCTCATCATCATCATTGT
TTTCCTTGTCATTAACTATCATCAGCGTGTCTATCACAACCGCCAGAGACTGGACATGGAAG
ATCCCTCATGTGAGATGTGTCTCTCCAAAGACAAGACGCTCCAGGATCTTGTCTACGATCTC
TCCACCTCAGGGTCTGGCTCAGGGTTACCCCTCTTTGTCCAGCGCACAGTGGCCCGAACCAT
CGTTTTACAAGAGATTATTGGCAAGGGTCGGTTTGGGGAAGTATGGCGGGGCCGCTGGAGGG
GTGGTGATGTGGCTGTGAAAATATTCTCTTCTCGTGAAGAACGGTCTTGGTTCAGGGAAGCA
GAGATATACCAGACGGTCATGCTGCGCCATGAAAACATCCTTGGATTTATTGCTGCTGACAA
TAAAGCAGACTGCTCATTCCTCACATTGCCATGGGAAGTTGTAATGGTCTCTGCTGCCCCCA
AGCTGAGGAGCCTTAGACTCCAATACAAGGGAGGAAGGGGAAGAGCAAGATTTTTATTCCCA
CTGAATAATGGCACCTGGACACAGCTGTGGCTTGTTTCTGACTATCATGAGCACGGGTCCCT
GTTTGATTATCTGAACCGGTACACAGTGACAATTGAGGGGATGATTAAGCTGGCCTTGTCTG
CTGCTAGTGGGCTGGCACACCTGCACATGGAGATCGTGGGCACCCAAGGGAAGCCTGGAATT
GCTCATCGAGACTTAAAGTCAAAGAACATTCTGGTGAAGAAAAATGGCATGTGTGCCATAGC
AGACCTGGGCCTGGCTGTCCGTCATGATGCAGTCACTGACACCATTGACATTGCCCCGAATC
AGAGGGTGGGGACCAAACGATACATGGCCCCTGAAGTACTTGATGAAACCATTAATATGAAA
CACTTTGACTCCTTTAAATGTGCTGATATTTATGCCCTCGGGCTTGTATATTGGGAGATTGC
TCGAAGATGCAATTCTGGAGGAGTCCATGAAGAATATCAGCTGCCATATTACGACTTAGTGC
CCTCTGACCCTTCCATTGAGGAAATGCGAAAGGTTGTATGTGATCAGAAGCTGCGTCCCAAC
ATCCCCAACTGGTGGCAGAGTTATGAGGCACTGCGGGTGATGGGGAAGATGATGCGAGAGTG
TTGGTATGCCAACGGCGCAGCCCGCCTGACGGCCCTGCGCATCAAGAAGACCCTCTCCCAGC
TCAGCGTGCAGGAAGACGTGAAGATC (SEQ ID NO: 85)

A nucleic acid sequence encoding the extracelluar ALK4 polypeptide (isoform C) is as follows:

TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTA
CACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACC
ATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTACTGCCTG

AGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGATCGACTT
GAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAG
(SEQ ID NO: 86)

In certain embodiments, the disclosure relates to 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.*, 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, 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, 104, 106, 403, or 404. In some embodiments, 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, 104, 106, 403, or 404.

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

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

61 **TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE LPTTVKSSPG**

121 **LGPVEL**AAVI AGPVCFVCIS LMLMVYICHN RTVIHHRVPN EEDPSLDRPF ISEGTTLKDL

181 IYDMTTSGSG SGLPLLVQRT IARTIVLQES IGKGRFGEVW RGKWRGEEVA VKIFSSREER

241 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS DYHEHGSLFD YLNRYTVTVE

301 GMIKLALSTA SGLAHLHMEI VGTQGKPAIA HRDLKSKNIL VKKNGTCCIA DLGLAVRHDS

361 ATDTIDIAPN HRVGTKRYMA PEVLDDSINM KHFESFKRAD IYAMGLVFWE IARRCSIGGI

421 HEDYQLPYYD LVPSDPSVEE MRKVVCEQKL RPNIPNRWQS CEALRVMAKI MRECWYANGA

481 ARLTALRIKK TLSQLSQQEG IKM (SEQ ID NO: 30)

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

The processed extracellular ALK5 polypeptide sequence is as follows:

AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVC APSSKTGSVTTTYCCNQDHCNKIELPTTVKSSPGLGPVEL (SEQ ID NO: 31)

A nucleic acid sequence encoding the ALK5 precursor protein is shown 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 GGCGGCGGCG**GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTA CAAAAGACAATTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACA GACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCC GTTTGTATGTGCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGG ACCATTGCAATAAAATAGAACTTCCAACTACTGTAAAGTCATCACCTGGCCTTGGTCCTGTG GAACTG**GCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACTCATGTTGATGGT CTATATCTGCCACAACCGCACTGTCATTCACCATCGAGTGCCAAATGAAGAGGACCCTTCAT

TAGATCGCCCTTTTATTTCAGAGGGTACTACGTTGAAAGACTTAATTTATGATATGACAACG
TCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAACAATTGCGAGAACTATTGTGTT
ACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGGGGAGAAG
AAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTCGTGGTTCCGTGAGGCAGAGATT
TATCAAACTGTAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGCAGACAATAAAGA
CAATGGTACTTGGACTCAGCTCTGGTTGGTGTCAGATTATCATGAGCATGGATCCCTTTTTG
ATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAAACTTGCTCTGTCCACGGCG
AGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGAAAGCCAGCCATTGCTCA
TAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACTTGCTGTATTGCAGACT
TAGGACTGGCAGTAAGACATGATTCAGCCACAGATACCATTGATATTGCTCCAAACCACAGA
GTGGGAACAAAAAGGTACATGGCCCCTGAAGTTCTCGATGATTCCATAAATATGAAACATTT
TGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCTGGGAAATTGCTCGAC
GATGTTCCATTGGTGGAATTCATGAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCT
GACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAGGCCAAATATCCC
AAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGAGAGAATGTTGGT
ATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATTAAGAAAACATTATCGCAACTCAGT
CAACAGGAAGGCATCAAAATG (SEQ ID NO: 32)

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

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
TTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTA
TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
GCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
TAAAATAGAACTTCCAACTACTGTAAAGTCATCACCTGGCCTTGGTCCTGTGGAACTG
(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 AAAA**AALLPG ATALQCFCHL CTKDNFTCVT**
DGLCFVSVTE
61 **TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE**
LPTTGPFSVK
121 **SSPGLGPVEL** AAVIAGPVCF VCISLMLMVY TCHNRTVIHH RVPNEEDPSL
DRPFISEGTT

181 LKDLIYDMTT SGSGSGLPLL VQRTIARTIV LQESIGKGRF GEVWRGKWRG
EEVAVKIFSS
241 REERSWFREA EIYQTVMLRH ENILGFIAAD NKDNGTWTQL WLVSDYHEHG
SLFDYLNRYT
301 VTVEGMIKLA LSTASGLAHL HMEIVGTQGK PAIAHRDLKS KNILVKKNGT
CCIADLGLAV
361 RHDSATDTID IAPNHRVGTK RYMAPEVLDD SINMKHFESF KRADIYAMGL
VFWEIARRCS
421 IGGIHEDYQL PYYDLVPSDP SVEEMRKVVC EQKLRPNIPN RWQSCEALRV
MAKIMRECWY
481 ANGAARLTAL RIKKTLSQLS QQEGIKM (SEQ ID NO: 87)

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

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

AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVC
APSSKTGSVTTTYCCNQDHCNKIELPTTGPFSVKSSPGLGPVEL (SEQ ID NO: 88)

A nucleic acid sequence encoding human ALK5 precursor protein (isoform 2) is shown 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.

ATGGAGGCGGCGGTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGC
GGCGGCGGCG**GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTA**
CAAAAGACAATTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACA
GACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCC
GTTTGTATGTGCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGG
ACCATTGCAATAAAATAGAACTTCCAACTACTGGCCCTTTTTCAGTAAAGTCATCACCTGGC
CTTGGTCCTGTGGAACTGGCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACT
CATGTTGATGGTCTATATCTGCCACAACCGCACTGTCATTCACCATCGAGTGCCAAATGAAG
AGGACCCTTCATTAGATCGCCCTTTTATTTCAGAGGGTACTACGTTGAAAGACTTAATTTAT
GATATGACAACGTCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAACAATTGCGAG
AACTATTGTGTTACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGT
GGCGGGGAGAAGAAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTCGTGGTTCCGT
GAGGCAGAGATTTATCAAACTGTAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGC

AGACAATAAAGACAATGGTACTTGGACTCAGCTCTGGTTGGTGTCAGATTATCATGAGCATG
GATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAAACTTGCT
CTGTCCACGGCGAGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGAAAGCC
AGCCATTGCTCATAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACTTGCT
GTATTGCAGACTTAGGACTGGCAGTAAGACATGATTCAGCCACAGATACCATTGATATTGCT
CCAAACCACAGAGTGGGAACAAAAAGGTACATGGCCCCTGAAGTTCTCGATGATTCCATAAA
TATGAAACATTTTGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCTGGG
AAATTGCTCGACGATGTTCCATTGGTGGAATTCATGAAGATTACCAACTGCCTTATTATGAT
CTTGTACCTTCTGACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAG
GCCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGA
GAGAATGTTGGTATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATTAAGAAAACATTA
TCGCAACTCAGTCAACAGGAAGGCATCAAAATG (SEQ ID NO: 89)

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

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
TTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTA
TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
GCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
TAAAATAGAACTTCCAACTACTGGCCCTTTTTCAGTAAAGTCATCACCTGGCCTTGGTCCTG
TGGAACTG (SEQ ID NO: 90)

In certain embodiments, the disclosure relates to 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.*, heteromultimer complexes comprising an ALK5 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK5). In other preferred embodiments, ALK5 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, 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% identical to the amino acid sequence of SEQ ID NO: 30, 31, 87, or 88. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one ALK5 polypeptide

that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 30, 31, 87, or 88.

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

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

1 MLLRSAGKLN VGT**KKEDGES TAPTPRPKVL RCKCHHHCPE DSVNNICSTD**
GYCFTMIEED
61 **DSGLPVVTSG CLGLEGSDFQ CRDTPIPHQR RSIECCTERN ECNKDLHPTL**
PPLKNRDFVD
121 **GPIHHR**ALLI SVTVCSLLLV LIILFCYFRY KRQETRPRYS IGLEQDETYI
PPGESLRDLI
181 EQSQSSGSGS GLPLLVQRTI AKQIQMVKQI GKGRYGEVWM GKWRGEKVAV
KVFFTTEEAS
241 WFRETEIYQT VLMRHENILG FIAADIKGTG SWTQLYLITD YHENGSLYDY
LKSTTLDAKS
301 MLKLAYSSVS GLCHLHTEIF STQGKPAIAH RDLKSKNILV KKNGTCCIAD
LGLAVKFISD
361 TNEVDIPPNT RVGTKRYMPP EVLDESLNRN HFQSYIMADM YSFGLILWEV
ARRCVSGGIV
421 EEYQLPYHDL VPSDPSYEDM REIVCIKKLR PSFPNRWSSD ECLRQMGKLM
TECWAHNPAS

481 RLTALRVKKT LAKMSESQDI KL (SEQ ID NO: 34)

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

The processed extracellular ALK6 polypeptide sequence is as follows:

KKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMIEEDDSGLPVVTSGCLGLE GSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFVDGPIHHR (SEQ ID NO: 35)

A nucleic acid sequence encoding the ALK6 precursor protein is shown 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 AGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATGCCACCACCATTGTCCAGAAGACT CAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCACGATGATAGAAGAGGATGACTCT GGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAAGGCTCAGATTTTCAGTGTCGGGA CACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAAGGAACGAATGTAATA AAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTTGATGGACCTATACAC CACAGG**GCTTTACTTATATCTGTGACTGTCTGTAGTTTGCTCTTGGTCCTTATCATATTATT TTGTTACTTCCGGTATAAAAGACAAGAAACCAGACCTCGATACAGCATTGGGTTAGAACAGG ATGAAACTTACATTCCTCCTGGAGAATCCCTGAGAGACTTAATTGAGCAGTCTCAGAGCTCA GGAAGTGGATCAGGCCTCCCTCTGCTGGTCCAAAGGACTATAGCTAAGCAGATTCAGATGGT GAAACAGATTGGAAAAGGTCGCTATGGGGAAGTTTGGATGGGAAAGTGGCGTGGCGAAAAGG TAGCTGTGAAAGTGTTCTTCACCACAGAGGAAGCCAGCTGGTTCAGAGAGACAGAAATATAT CAGACAGTGTTGATGAGGCATGAAAACATTTTGGGTTTCATTGCTGCAGATATCAAAGGGAC AGGGTCCTGGACCCAGTTGTACCTAATCACAGACTATCATGAAAATGGTTCCCTTTATGATT ATCTGAAGTCCACCACCCTAGACGCTAAATCAATGCTGAAGTTAGCCTACTCTTCTGTCAGT GGCTTATGTCATTTACACACAGAAATCTTTAGTACTCAAGGCAAACCAGCAATTGCCCATCG AGATCTGAAAAGTAAAAACATTCTGGTGAAGAAAAATGGAACTTGCTGTATTGCTGACCTGG GCCTGGCTGTTAAATTTATTAGTGATACAAATGAAGTTGACATACCACCTAACACTCGAGTT GGCACCAAACGCTATATGCCTCCAGAAGTGTTGGACGAGAGCTTGAACAGAAATCACTTCCA GTCTTACATCATGGCTGACATGTATAGTTTTGGCCTCATCCTTTGGGAGGTTGCTAGGAGAT GTGTATCAGGAGGTATAGTGGAAGAATACCAGCTTCCTTATCATGACCTAGTGCCCAGTGAC CCCTCTTATGAGGACATGAGGGAGATTGTGTGCATCAAGAAGTTACGCCCCTCATTCCCAAA

CCGGTGGAGCAGTGATGAGTGTCTAAGGCAGATGGGAAAACTCATGACAGAATGCTGGGCTC
ACAATCCTGCATCAAGGCTGACAGCCCTGCGGGTTAAGAAAACACTTGCCAAAATGTCAGAG
TCCCAGGACATTAAACTC (SEQ ID NO: 36)

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

AAGAAAGAGGATGGTGAGAGTACAGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATG
CCACCACCATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCA
CGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAA
GGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTG
CACAGAAAGGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAG
ATTTTGTTGATGGACCTATACACCACAGG (SEQ ID NO: 37)

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

1 MGWLEELNWQ LHIFLLILLS MHTRA**NFLDN MLLRSAGKLN VGTKKEDGES**
TAPTPRPKVL
61 **RCKCHHHCPE DSVNNICSTD GYCFTMIEED DSGLPVVTSG CLGLEGSDFQ**
CRDTPIPHQR
121 **RSIECCTERN ECNKDLHPTL PPLKNRDFVD GPIHHR**ALLI SVTVCSLLLV
LIILFCYFRY
181 KRQETRPRYS IGLEQDETYI PPGESLRDLI EQSQSSGSGS GLPLLVQRTI
AKQIQMVKQI
241 GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT VLMRHENILG
FIAADIKGTG
301 SWTQLYLITD YHENGSLYDY LKSTTLDAKS MLKLAYSSVS GLCHLHTEIF
STQGKPAIAH
361 RDLKSKNILV KKNGTCCIAD LGLAVKFISD TNEVDIPPNT RVGTKRYMPP
EVLDESLNRN
421 HFQSYIMADM YSFGLILWEV ARRCVSGGIV EEYQLPYHDL VPSDPSYEDM
REIVCIKKLR
481 PSFPNRWSSD ECLRQMGKLM TECWAHNPAS RLTALRVKKT LAKMSESQDI KL (SEQ
ID NO: 91)

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

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

NFLDNMLLRSAGKLNVGTKKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMI
EEDDSGLPVVTSGCLGLEGSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFV
DGPIHHR (SEQ ID NO: 92)

A nucleic acid sequence encoding human ALK6 precursor protein (isoform 2) is shown 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.

ATGGGTTGGCTGGAAGAACTAAACTGGCAGCTTCACATTTTCTTGCTCATTCTTCTCTCTAT
GCACACAAGGGCA**AACTTCCTTGATAACATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGG**
GCACCAAGAAAGAGGATGGTGAGAGTACAGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGT
AAATGCCACCACCATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTG
TTTCACGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGAC
TAGAAGGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAA
TGCTGCACAGAAAGGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAA
CAGAGATTTTGTTGATGGACCTATACACCACAGGGCTTTACTTATATCTGTGACTGTCTGTA
GTTTGCTCTTGGTCCTTATCATATTATTTTGTTACTTCCGGTATAAAAGACAAGAAACCAGA
CCTCGATACAGCATTGGGTTAGAACAGGATGAAACTTACATTCCTCCTGGAGAATCCCTGAG
AGACTTAATTGAGCAGTCTCAGAGCTCAGGAAGTGGATCAGGCCTCCCTCTGCTGGTCCAAA
GGACTATAGCTAAGCAGATTCAGATGGTGAAACAGATTGGAAAAGGTCGCTATGGGGAAGTT
TGGATGGGAAAGTGGCGTGGCGAAAAGGTAGCTGTGAAAGTGTTCTTCACCACAGAGGAAGC
CAGCTGGTTCAGAGAGACAGAAATATATCAGACAGTGTTGATGAGGCATGAAAACATTTTGG
GTTTCATTGCTGCAGATATCAAAGGGACAGGGTCCTGGACCCAGTTGTACCTAATCACAGAC
TATCATGAAAATGGTTCCCTTTATGATTATCTGAAGTCCACCACCCTAGACGCTAAATCAAT
GCTGAAGTTAGCCTACTCTTCTGTCAGTGGCTTATGTCATTTACACACAGAAATCTTTAGTA
CTCAAGGCAAACCAGCAATTGCCCATCGAGATCTGAAAAGTAAAAACATTCTGGTGAAGAAA
AATGGAACTTGCTGTATTGCTGACCTGGGCCTGGCTGTTAAATTTATTAGTGATACAAATGA
AGTTGACATACCACCTAACACTCGAGTTGGCACCAAACGCTATATGCCTCCAGAAGTGTTGG
ACGAGAGCTTGAACAGAAATCACTTCCAGTCTTACATCATGGCTGACATGTATAGTTTTGGC
CTCATCCTTTGGGAGGTTGCTAGGAGATGTGTATCAGGAGGTATAGTGGAAGAATACCAGCT
TCCTTATCATGACCTAGTGCCCAGTGACCCCTCTTATGAGGACATGAGGGAGATTGTGTGCA
TCAAGAAGTTACGCCCCTCATTCCCAAACCGGTGGAGCAGTGATGAGTGTCTAAGGCAGATG

GGAAAACTCATGACAGAATGCTGGGCTCACAATCCTGCATCAAGGCTGACAGCCCTGCGGGT
TAAGAAAACACTTGCCAAAATGTCAGAGTCCCAGGACATTAAACTC (SEQ ID NO: 93)

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

AACTTCCTTGATAACATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACCAAGAAAGA
GGATGGTGAGAGTACAGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATGCCACCACC
ATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCACGATGATA
GAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAAGGCTCAGA
TTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAA
GGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTT
GATGGACCTATACACCACAGG (SEQ ID NO: 94)

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

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

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

Four 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 PME**LAIIITV

121 PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL IYDVTASGSG

181 SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ

241 TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA

301 SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN

361 PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD

421 MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK

481 TISQLCVKED CKA (SEQ ID NO: 38)

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

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

ELSPGLKCVCLLCDSSNFTCQTEGACWASVMLTNGKEQVIKSCVSLPELNAQVFCHSSNNVT KTECCFTDFCNNITLHLPTASPNAPKLGPME (SEQ ID NO: 39)

A nucleic acid sequence encoding human ALK7 isoform 1 precursor protein is shown below (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.

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCC**GA**
GCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAA
CAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCC
TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAA
AACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATCAC
CAAATGCCCCAAAACTTGGACCCATGGAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTC
CTGTCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTGCTCCTACAGGAA
GAAAAAGAGACCAAATGTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTGGAAAAA
CTCTGAAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTG
GTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGG
TGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAG
ATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAAC
ATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGGCTGGT
ATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGG
CTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATT
GTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGT
GAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATAC
TGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAA
ATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTC
TGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGT
ACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTT
GTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCG
AGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTC
TTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 40)

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

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCA
AACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAAT
CCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC

AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATC
ACCAAATGCCCCAAAACTTGGACCCATGGAG (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 TASPNAPKLG**
61 **PME**LAIIITV PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL
121 IYDVTASGSG SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER
181 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA
241 GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS
301 ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI
361 VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA
421 ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 301)

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 CTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAA CACTGCACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATGGAGCTGGCCATCATTATT ACTGTGCCTGTTTGCCTCCTGTCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTG CTCCTACAGGAAGAAAAAGAGACCAAATGTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTG GAAAAACTCTGAAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTG GTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGT

GTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTT
GGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCT
GCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTC
CTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTG
CTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGA
GACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGC
TGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGT
ATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGAC
ATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGA
GTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTT
GTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGG
AGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGAC
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
CACTGCACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATGGAG (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.

1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV
MLTNGKEQVI
61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TGLPLLVQRT
IARTIVLQEI
121 VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ TVMLRHENIL
GFIAADNKDN
181 GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA SGLAHLHMEI
VGTQGKPAIA
241 HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA
PEMLDDTMNV
301 NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD MVPSDPSIEE
MRKVVCDQKF
361 RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK TISQLCVKED CKA

(SEQ ID NO: 305)

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

1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN

61 VTKTECCFTD FCNNITLHLP TGLPLLVQRT IARTIVLQEI VGKGRFGEVW HCRWCGEDVA

121 VKIFSSRDER SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD

181 YLNRNIVTVA GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA

241 DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE

301 IARRCSVGGI VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI

361 MRECWYANGA ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 306)

A nucleic acid sequence encoding 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.

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGAGCTCTC
GCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCAT
GTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAA
CTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTT
TTGCAACAACATAACACTGCACCTTCCAACAGGTCTACCTCTGTTGGTTCAAAGGACAATTGCAAGGA
CGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGG
GAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTA
CCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAA
CTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGA
AATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCA
TATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCT
TAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTG
AACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGA

TGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTT
ACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGAC
ATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAG
TATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGT
ATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAA
GAAGACTGCAAAGCC (SEQ ID NO: 307)

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

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGA
AGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCC
TTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTC
ACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGGTCTACCTCTGTTGGTTCAAAGGACAAT
TGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGAT
GGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCA
GAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGA
TAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATT
TGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCA
CACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAA
GAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATT
CAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAA
ATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGG
TCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTT
ATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTT
CGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGA
GTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTT
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.

1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV
MLTNGKEQVI
61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TDNGTWTQLW
LVSEYHEQGS
121 LYDYLNRNIV TVAGMIKLAL SIASGLAHLH MEIVGTQGKP AIAHRDIKSK
NILVKKCETC

181 AIADLGLAVK HDSILNTIDI PQNPKVGTKR YMAPEMLDDT MNVNIFESFK RADIYSVGLV

241 YWEIARRCSV GGIVEEYQLP YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM

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

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 68:1719-1726). N-terminal variants of SEQ ID NO: 310 are predicted as described below.

1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN

61 VTKTECCFTD FCNNITLHLP TDNGTWTQLW LVSEYHEQGS LYDYLNRNIV TVAGMIKLAL

121 SIASGLAHLH MEIVGTQGKP AIAHRDIKSK NILVKKCETC AIADLGLAVK HDSILNTIDI

181 PQNPKVGTKR YMAPEMLDDT MNVNIFESFK RADIYSVGLV YWEIARRCSV GGIVEEYQLP

240 YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM GRIMRECWYA NGAARLTALR

301 IKKTISQLCV KEDCKA (SEQ ID NO: 310)

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 solid underline.

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGAGCTCTC
GCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCAT
GTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAA
CTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTT
TTGCAACAACATAACACTGCACCTTCCAACAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTG
AATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATC
AAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAA
ACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCA
TAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCT
AAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGA
GTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAG

TCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAG
GAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGA
AGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTG
CTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCCTAA (SEQ
ID NO: 311)

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

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGA
AGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCC
TTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTC
ACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGATAATGGAACTTGGACTCAACTTTGGCT
GGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTG
GAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACA
CAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAAC
TTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTC
AGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAAT
ATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAG
GTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCT
CGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAA
AGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCG
CCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCCTAA
(SEQ ID NO: 312)

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

1 LKCVCLLCDS SNFTCQTEGA CWASVMLTNG KEQVIKSCVS LPELNAQVFC
HSSNNVTKTE

61 CCFTDFCNNI TLHLPTASPN APKLGPME (SEQ ID NO: 313)

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

In certain embodiments, the disclosure relates to 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.*, 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, 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, 112, 114, 301, 302, 305, 306, 309, 310, 313, 405, or 406. In some embodiments, 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, 112, 114, 301, 302, 305, 306, 309, 310, 313, 405, or 406.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ActRIIB heteromultimer complexes of the disclosure comprise at least one ALK1 polypeptide that comprises, consists of, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ActRIIB heteromultimers of the disclosure comprises at least one ActRIIB polypeptide that comprises, consists, or consists essentially a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK1:ActRIIB heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In certain preferred embodiments, ActRIIB polypeptides of the disclosure do not comprise an acidic amino acid (e.g., a naturally

occurring D or E amino acid or artificially acidic amino acid). Preferably, ALK1:ActRIIB heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ActRIIB). In other embodiments, ALK1:ActRIIB heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ActRIIB heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ActRIIB homomultimers). ALK1:ActRIIB heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ActRIIB heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:ActRIIB heteromultimer complexes of the disclosure comprise at least one ALK2 polypeptide that comprises, consists of, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:ActRIIB heteromultimers of the disclosure comprises at least one ActRIIB polypeptide that comprises, consists, or consists essentially a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK2:ActRIIB heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In certain preferred embodiments, ActRIIB

polypeptides of the disclosure do not comprise an acidic amino acid (e.g., a naturally occurring D or E amino acid or artificially acidic amino acid). Preferably, ALK2:ActRIIB heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or ActRIIB). In other embodiments, ALK2:ActRIIB heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:ActRIIB heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and ActRIIB homomultimers). ALK2:ActRIIB heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:ActRIIB heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK3:ActRIIB heteromultimer complexes of the disclosure comprise at least one ALK3 polypeptide that comprises, consists of, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. In some embodiments, ALK3:ActRIIB heteromultimers of the disclosure comprises at least one ActRIIB polypeptide that comprises, consists, or consists essentially a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK3:ActRIIB heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100,

102, 153, 154, 401, 402, 453, and 454. In certain preferred embodiments, ActRIIB polypeptides of the disclosure do not comprise an acidic amino acid (c.g., a naturally occurring D or E amino acid or artificially acidic amino acid). Preferably, ALK3:ActRIIB heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or ActRIIB). In other embodiments, ALK3:ActRIIB heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:ActRIIB heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and ActRIIB homomultimers). ALK3:ActRIIB heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:ActRIIB heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK4:ActRIIB heteromultimer complexes of the disclosure comprise at least one ALK4 polypeptide that comprises, consists of, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NO: 26 or 83. In some embodiments, ALK4:ActRIIB heteromultimers of the disclosure comprises at least one ActRIIB polypeptide that comprises, consists, or consists essentially a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK4:ActRIIB heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%,

98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In certain preferred embodiments, ActRIIB polypeptides of the disclosure do not comprise an acidic amino acid (e.g., a naturally occurring D or E amino acid or artificially acidic amino acid). Preferably, ALK4:ActRIIB heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK4 and/or ActRIIB). In other embodiments, ALK4:ActRIIB heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK4:ActRIIB heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK4 and ActRIIB homomultimers). ALK4:ActRIIB heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK4:ActRIIB heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK5:ActRIIB heteromultimer complexes of the disclosure comprise at least one ALK5 polypeptide that comprises, consists of, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NO: 30 or 87. In some embodiments, ALK5:ActRIIB heteromultimers of the disclosure comprises at least one ActRIIB polypeptide that comprises, consists, or consists essentially a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK5:ActRIIB heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a

sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In certain preferred embodiments, ActRIIB polypeptides of the disclosure do not comprise an acidic amino acid (e.g., a naturally occurring D or E amino acid or artificially acidic amino acid). Preferably, ALK5:ActRIIB heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK5 and/or ActRIIB). In other embodiments, ALK5:ActRIIB heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK5:ActRIIB heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK5 and ActRIIB homomultimers). ALK5:ActRIIB heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK5:ActRIIB heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK6:ActRIIB heteromultimer complexes of the disclosure comprise at least one ALK6 polypeptide that comprises, consists of, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. In some embodiments, ALK6:ActRIIB heteromultimers of the disclosure comprises at least one ActRIIB polypeptide that comprises, consists, or consists essentially a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK6:ActRIIB

heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In certain preferred embodiments, ActRIIB polypeptides of the disclosure do not comprise an acidic amino acid (e.g., a naturally occurring D or E amino acid or artificially acidic amino acid). Preferably, ALK6:ActRIIB heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK6 and/or ActRIIB). In other embodiments, ALK6:ActRIIB heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK6:ActRIIB heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK6 and ActRIIB homomultimers). ALK6:ActRIIB heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK6:ActRIIB heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK7:ActRIIB heteromultimer complexes of the disclosure comprise at least one ALK7 polypeptide that comprises, consists of, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-92 of SEQ ID Nos: 38, 305, and 309. In some embodiments, ALK7:ActRIIB heteromultimers of the disclosure comprises at least one ActRIIB polypeptide that comprises, consists, or consists essentially a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical

to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK7:ActRIIB heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454. In certain preferred embodiments, ActRIIB polypeptides of the disclosure do not comprise an acidic amino acid (e.g., a naturally occurring D or E amino acid or artificially acidic amino acid). Preferably, ALK7:ActRIIB heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK7 and/or ActRIIB). In other embodiments, ALK7:ActRIIB heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK7:ActRIIB heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK7 and ActRIIB homomultimers). ALK7:ActRIIB heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK7:ActRIIB heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ActRIIA heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ActRIIA heteromultimers of the disclosure comprises at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%,

98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. Preferably, ALK1:ActRIIA heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ActRIIA). In other preferred embodiments, ALK1:ActRIIA heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ActRIIA heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ActRIIA homomultimers). ALK1:ActRIIA heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ActRIIA heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:ActRIIA heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:ActRIIA heteromultimers of the disclosure comprises at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. Preferably, ALK2:ActRIIA heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or ActRIIA). In other preferred embodiments, ALK2:ActRIIA heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:ActRIIA heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and ActRIIA homomultimers). ALK2:ActRIIA heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:ActRIIA heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK3:ActRIIA heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. In some embodiments, ALK3:ActRIIA heteromultimers of the disclosure comprises at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. Preferably, ALK3:ActRIIA heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or ActRIIA). In other preferred embodiments, ALK3:ActRIIA heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE,

nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:ActRIIA heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and ActRIIA homomultimers). ALK3:ActRIIA heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:ActRIIA heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK4:ActRIIA heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NO: 26 or 83. In some embodiments, ALK4:ActRIIA heteromultimers of the disclosure comprises at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. Preferably, ALK4:ActRIIA heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK4 and/or ActRIIA). In other preferred embodiments, ALK4:ActRIIA heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK4:ActRIIA heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK4 and ActRIIA homomultimers). ALK4:ActRIIA heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers,

heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK4:ActRIIA heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK5:ActRIIA heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NO: 30 or 87. In some embodiments, ALK5:ActRIIA heteromultimers of the disclosure comprises at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. Preferably, ALK5:ActRIIA heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK5 and/or ActRIIA). In other preferred embodiments, ALK5:ActRIIA heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK5:ActRIIA heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK5 and ActRIIA homomultimers). ALK5:ActRIIA heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK5:ActRIIA heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIA polypeptide, which includes fragments, functional variants,

and modified forms thereof. In some embodiments, ALK6:ActRIIA heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. In some embodiments, ALK6:ActRIIA heteromultimers of the disclosure comprises at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. Preferably, ALK6:ActRIIA heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK6 and/or ActRIIA). In other preferred embodiments, ALK6:ActRIIA heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK6:ActRIIA heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK6 and ActRIIA homomultimers). ALK6:ActRIIA heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK6:ActRIIA heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK7:ActRIIA heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ

ID NO: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-93 of SEQ ID Nos: 38, 305, or 309. In some embodiments, ALK7:ActRIIA heteromultimers of the disclosure comprises at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. Preferably, ALK7:ActRIIA heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK7 and/or ActRIIA). In other preferred embodiments, ALK7:ActRIIA heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK7:ActRIIA heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK7 and ActRIIA homomultimers). ALK7:ActRIIA heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK7:ActRIIA heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:TGFBRII heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:TGFBRII heteromultimer complexes of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%,

98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ALK1:TGFBRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or TGFBRII). In other preferred embodiments, ALK1:TGFBRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:TGFBRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and TGFBRII homomultimers). ALK1:TGFBRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:TGFBRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:TGFBRII heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:TGFBRII heteromultimer complexes of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ALK2:TGFBRII heteromultimer complexes of the present disclosure are soluble (*e.g.*,

comprise an extracellular domain of ALK2 and/or TGFBRII). In other preferred embodiments, ALK2:TGFBRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:TGFBRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and TGFBRII homomultimers). ALK2:TGFBRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:TGFBRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK3:TGFBRII heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. In some embodiments, ALK3:TGFBRII heteromultimer complexes of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ALK3:TGFBRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or TGFBRII). In other preferred embodiments, ALK3:TGFBRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7,

BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:TGFBRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and TGFBRII homomultimers). ALK3:TGFBRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:TGFBRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK4:TGFBRII heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NO: 26 or 83. In some embodiments, ALK4:TGFBRII heteromultimer complexes of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ALK4:TGFBRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK4 and/or TGFBRII). In other preferred embodiments, ALK4:TGFBRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin,

persephin, MIS, and Lefty). In some embodiments, ALK4:TGFBRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK4 and TGFBRII homomultimers). ALK4:TGFBRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK4:TGFBRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK5:TGFBRII heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NO: 30 or 87. In some embodiments, ALK5:TGFBRII heteromultimer complexes of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ALK5:TGFBRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK5 and/or TGFBRII). In other preferred embodiments, ALK5:TGFBRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK5:TGFBRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK5 and TGFBRII homomultimers). ALK5:TGFBRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers,

heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK5:TGFBRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK6:TGFBRII heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. In some embodiments, ALK6:TGFBRII heteromultimer complexes of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ALK6:TGFBRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK6 and/or TGFBRII). In other preferred embodiments, ALK6:TGFBRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK6:TGFBRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK6 and TGFBRII homomultimers). ALK6:TGFBRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK6:TGFBRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK7:TGFBRII heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-93 of SEQ ID Nos: 38, 305, or 309. In some embodiments, ALK7:TGFBRII heteromultimer complexes of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ALK7:TGFBRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK7 and/or TGFBRII). In other preferred embodiments, ALK7:TGFBRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK7:TGFBRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK7 and TGFBRII homomultimers). ALK7:TGFBRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK7:TGFBRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants,

and modified forms thereof. In some embodiments, ALK1:MISRII heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:MISRII heteromultimer complexes of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID Nos: 50, 75, or 79. Preferably, ALK1:MISRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or MISRII). In other preferred embodiments, ALK1:MISRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:MISRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and MISRII homomultimers). ALK1:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:MISRII heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID

NO: 18. In some embodiments, ALK2:MISRII heteromultimer complexes of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID Nos: 50, 75, or 79. Preferably, ALK2:MISRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or MISRII). In other preferred embodiments, ALK2:MISRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:MISRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and MISRII homomultimers). ALK2:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK3:MISRII heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. In some embodiments, ALK3:MISRII heteromultimer complexes of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID

Nos: 50, 75, or 79. Preferably, ALK3:MISRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or MISRII). In other preferred embodiments, ALK3:MISRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:MISRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and MISRII homomultimers). ALK3:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK4:MISRII heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NO: 26 or 83. In some embodiments, ALK4:MISRII heteromultimer complexes of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID Nos: 50, 75, or 79. Preferably, ALK4:MISRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK4 and/or MISRII). In other preferred embodiments, ALK4:MISRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7,

BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK4:MISRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK4 and MISRII homomultimers). ALK4:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK4:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK5:MISRII heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NOs: 30 or 87. In some embodiments, ALK5:MISRII heteromultimer complexes of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID Nos: 50, 75, or 79. Preferably, ALK5:MISRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK5 and/or MISRII). In other preferred embodiments, ALK5:MISRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK5:MISRII

heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK5 and MISRII homomultimers). ALK5:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK5:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK6:MISRII heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 SEQ ID NO: 91. In some embodiments, ALK6:MISRII heteromultimer complexes of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID Nos: 50, 75, or 79. Preferably, ALK6:MISRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK6 and/or MISRII). In other preferred embodiments, ALK6:MISRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK6:MISRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK6 and MISRII homomultimers). ALK6:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers

and further oligomeric structures. In certain preferred embodiments, ALK6:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK7:MISRII heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-93 of SEQ ID NOs: 38, 305, or 309. In some embodiments, ALK7:MISRII heteromultimer complexes of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID Nos: 50, 75, or 79. Preferably, ALK7:MISRII heteromultimer complexes of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK7 and/or MISRII). In other preferred embodiments, ALK7:MISRII heteromultimer complexes 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK7:MISRII heteromultimer complexes of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK7 and MISRII homomultimers). ALK7:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK7:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms

thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:BMPRII heteromultimer complexes of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:BMPRII heteromultimer complexes of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. Preferably, ALK1:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or BMPRII). In other preferred embodiments, ALK1:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and BMPRII homomultimers). ALK1:BMPRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:BMPRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:BMPRII heteromultimer complexes of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any

one of SEQ ID NO: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:BMPRII heteromultimer complexes of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. Preferably, ALK2:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or BMPRII). In other preferred embodiments, ALK2:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and BMPRII homomultimers). ALK2:BMPRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:BMPRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK3:BMPRII heteromultimer complexes of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. In some embodiments, ALK3:BMPRII heteromultimer complexes of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of

SEQ ID NO: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. Preferably, ALK3:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or BMPRII). In other preferred embodiments, ALK3:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and BMPRII homomultimers). ALK3:BMPRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:BMPRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK4:BMPRII heteromultimer complexes of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NOs: 26 or 83. In some embodiments, ALK4:BMPRII heteromultimer complexes of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. Preferably, ALK4:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK4 and/or BMPRII). In other preferred embodiments, ALK4:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK4:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK4 and BMPRII homomultimers). ALK4:BMPRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK4:BMPRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK5:BMPRII heteromultimer complexes of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NOs: 30 or 87. In some embodiments, ALK5:BMPRII heteromultimer complexes of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. Preferably, ALK5:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK5 and/or BMPRII). In other preferred embodiments, ALK5:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin

AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK5:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK5 and BMPRII homomultimers). ALK5:BMPRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK5:BMPRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK6:BMPRII heteromultimer complexes of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. In some embodiments, ALK6:BMPRII heteromultimer complexes of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. Preferably, ALK6:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK6 and/or BMPRII). In other preferred embodiments, ALK6:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK6:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer

complexes (*i.e.*, ALK6 and BMPRII homomultimers). ALK6:BMPRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK6:BMPRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK7:BMPRII heteromultimer complexes of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-92 of SEQ ID NOs: 38, 305, or 309. In some embodiments, ALK7:BMPRII heteromultimer complexes of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. Preferably, ALK7:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK7 and/or BMPRII). In other preferred embodiments, ALK6:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK7:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK7 and BMPRII homomultimers). ALK7:BMPRII heteromultimer complexes of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK7:BMPRII heteromultimer complexes of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ALK2 heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ALK2 heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. Preferably, ALK1:ALK2 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ALK2). In other preferred embodiments, ALK1:ALK2 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ALK2 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ALK2 homomultimers). ALK1:ALK2 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ALK2 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ALK2 heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%,

95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ALK2 heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. Preferably, ALK1:ALK2 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ALK2). In other preferred embodiments, ALK1:ALK2 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ALK2 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ALK2 homomultimers). ALK1:ALK2 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ALK2 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ALK3 heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ALK3 heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 22, 23, 115,

117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. Preferably, ALK1:ALK3 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ALK3). In other preferred embodiments, ALK1:ALK3 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ALK3 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ALK3 homomultimers). ALK1:ALK3 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ALK3 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ALK4 heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ALK4 heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NOs: 26 or 83. Preferably, ALK1:ALK4 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ALK4). In other preferred embodiments, ALK1:ALK4 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7,

BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ALK4 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ALK4 homomultimers). ALK1:ALK4 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ALK4 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ALK4 heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ALK4 heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NOs: 26 or 83. Preferably, ALK1:ALK4 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ALK4). In other preferred embodiments, ALK1:ALK4 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ALK4 heteromultimers of the disclosure have different

ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ALK4 homomultimers). ALK1:ALK4 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ALK4 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ALK5 heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ALK5 heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NOs: 30 or 87. Preferably, ALK1:ALK5 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ALK5). In other preferred embodiments, ALK1:ALK5 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ALK5 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ALK5 homomultimers). ALK1:ALK5 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ALK5 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ALK6 heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ALK6 heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. Preferably, ALK1:ALK6 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ALK6). In other preferred embodiments, ALK1:ALK6 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ALK6 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ALK6 homomultimers). ALK1:ALK6 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ALK6 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK1 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK1:ALK7 heteromultimers of the disclosure comprise at least one ALK1 polypeptide that comprises, consists, or consists

essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464 or amino acids 34-95 of SEQ ID NO: 14. In some embodiments, ALK1:ALK7 heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-92 of SEQ ID NOs: 38, 305, or 309. Preferably, ALK1:ALK7 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK1 and/or ALK7). In other preferred embodiments, ALK1:ALK7 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK1:ALK7 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK1 and ALK7 homomultimers). ALK1:ALK7 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK1:ALK7 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:ALK3 heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:ALK3 heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence

that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. Preferably, ALK2:ALK3 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or ALK3). In other preferred embodiments, ALK2:ALK3 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:ALK3 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and ALK3 homomultimers). ALK2:ALK3 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:ALK3 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:ALK4 heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:ALK4 heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NOs: 26 or 83. Preferably, ALK2:ALK4 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or ALK4). In other preferred embodiments, ALK2:ALK4 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:ALK4 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and ALK4 homomultimers). ALK2:ALK4 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:ALK4 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:ALK5 heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:ALK5 heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NOs: 30 or 87. Preferably, ALK2:ALK5 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or ALK5). In other preferred embodiments, ALK2:ALK5 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE,

nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:ALK5 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and ALK5 homomultimers). ALK2:ALK5 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:ALK5 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:ALK6 heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:ALK6 heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. Preferably, ALK2:ALK6 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or ALK6). In other preferred embodiments, ALK2:ALK6 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:ALK6 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and ALK6 homomultimers). ALK2:ALK6 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers

and further oligomeric structures. In certain preferred embodiments, ALK2:ALK6 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK2:ALK7 heteromultimers of the disclosure comprise at least one ALK2 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466 or amino acids 35-99 of SEQ ID NO: 18. In some embodiments, ALK2:ALK7 heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-92 of SEQ ID NOs: 38, 305, or 309. Preferably, ALK2:ALK7 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK2 and/or ALK7). In other preferred embodiments, ALK2:ALK7 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK2:ALK7 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK2 and ALK7 homomultimers). ALK2:ALK7 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK2:ALK7 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK4 polypeptide, which includes fragments, functional variants,

and modified forms thereof. In some embodiments, ALK3:ALK4 heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. In some embodiments, ALK3:ALK4 heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NOs: 26 or 83. Preferably, ALK3:ALK4 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or ALK4). In other preferred embodiments, ALK3:ALK4 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:ALK4 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and ALK4 homomultimers). ALK3:ALK4 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:ALK4 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK3:ALK5 heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID

NO: 22. In some embodiments, ALK3:ALK5 heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NOs: 30 or 87. Preferably, ALK3:ALK5 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or ALK5). In other preferred embodiments, ALK3:ALK5 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:ALK5 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and ALK5 homomultimers). ALK3:ALK5 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:ALK5 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK3:ALK6 heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. In some embodiments, ALK3:ALK6 heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or

amino acids 62-132 of SEQ ID NO: 91. Preferably, ALK3:ALK6 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or ALK6). In other preferred embodiments, ALK3:ALK6 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:ALK6 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and ALK6 homomultimers). ALK3:ALK6 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:ALK6 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK3:ALK7 heteromultimers of the disclosure comprise at least one ALK3 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468 or amino acids 61-130 of SEQ ID NO: 22. In some embodiments, ALK3:ALK7 heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-92 of SEQ ID NOs: 38, 305, or 309. Preferably, ALK3:ALK7 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK3 and/or ALK7). In other preferred embodiments, ALK3:ALK7 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7,

BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK3:ALK7 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK3 and ALK7 homomultimers). ALK3:ALK7 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK3:ALK7 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK4:ALK5 heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NOs: 26 or 83. In some embodiments, ALK4:ALK5 heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NOs: 30 or 87. Preferably, ALK4:ALK5 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK4 and/or ALK5). In other preferred embodiments, ALK4:ALK5 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK4:ALK5 heteromultimers of the disclosure have different

ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK4 and ALK5 homomultimers). ALK4:ALK5 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK4:ALK5 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK4:ALK6 heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NOs: 26 or 83. In some embodiments, ALK4:ALK6 heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. Preferably, ALK4:ALK6 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK4 and/or ALK6). In other preferred embodiments, ALK4:ALK6 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK4:ALK6 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK4 and ALK6 homomultimers). ALK4:ALK6 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK4:ALK6 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK4:ALK7 heteromultimers of the disclosure comprise at least one ALK4 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470 or amino acids 34-101 of SEQ ID NOs: 26 or 83. In some embodiments, ALK4:ALK7 heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-92 of SEQ ID NOs: 38, 305, or 309. Preferably, ALK4:ALK7 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK4 and/or ALK7). In other preferred embodiments, ALK4:ALK7 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK4:ALK7 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK4 and ALK7 homomultimers). ALK4:ALK7 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK4:ALK7 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK5:ALK6 heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists

essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NOs: 30 or 87. In some embodiments, ALK5:ALK6 heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. Preferably, ALK5:ALK6 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK5 and/or ALK6). In other preferred embodiments, ALK5:ALK6 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK5:ALK6 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK5 and ALK6 homomultimers). ALK5:ALK6 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK5:ALK6 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK5:ALK7 heteromultimers of the disclosure comprise at least one ALK5 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472 or amino acids 36-106 of SEQ ID NOs: 30 or 87. In some embodiments, ALK5:ALK7 heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists

essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-92 of SEQ ID NOs: 38, 305, or 309. Preferably, ALK5:ALK7 heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ALK5 and/or ALK7). In other preferred embodiments, ALK5:ALK7 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK5:ALK7 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK5 and ALK7 homomultimers). ALK5:ALK7 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK5:ALK7 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ALK6 polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ALK6:ALK7 heteromultimers of the disclosure comprise at least one ALK6 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474 or amino acids 32-102 of SEQ ID NO: 34 or amino acids 62-132 of SEQ ID NO: 91. In some embodiments, ALK6:ALK7 heteromultimers of the disclosure comprise at least one ALK7 polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476 or amino acids 28-92 of SEQ ID NOs: 38, 305, or 309. Preferably, ALK5:ALK7 heteromultimers of the present disclosure are soluble (*e.g.*,

comprise an extracellular domain of ALK6 and/or ALK7). In other preferred embodiments, ALK6:ALK7 heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ALK6:ALK7 heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ALK6 and ALK7 homomultimers). ALK6:ALK7 heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ALK6:ALK7 heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ActRIIA:ActRIIB heteromultimers of the disclosure comprise at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. In some embodiments, ActRIIA:ActRIIB heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454 or amino acids 29-109 of SEQ ID NO: 1 or 25-131 of SEQ ID NO: 1. Preferably, ActRIIA:ActRIIB heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ActRIIA and/or ActRIIB). In other preferred embodiments, ActRIIA:ActRIIB heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13,

GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ActRIIA:ActRIIB heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ActRIIA and ActRIIB homomultimers). ActRIIA:ActRIIB heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ActRIIA:ActRIIB heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ActRIIA:BMPRII heteromultimers of the disclosure comprise at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. In some embodiments, ActRIIA:BMPRII heteromultimers of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 and 71. Preferably, ActRIIA:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ActRIIA and/or BMPRII). In other preferred embodiments, ActRIIA:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ActRIIA:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer

complexes (*i.e.*, ActRIIA and BMPRII homomultimers). ActRIIA:BMPRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ActRIIA:BMPRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ActRIIA:TGFBRII heteromultimers of the disclosure comprise at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. In some embodiments, ActRIIA:TGFBRII heteromultimers of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ActRIIA:TGFBRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ActRIIA and/or BMPRII). In other preferred embodiments, ActRIIA:TGFBRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ActRIIA:TGFBRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ActRIIA and TGFBRII homomultimers). ActRIIA:TGFBRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ActRIIA:TGFBRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ActRIIA:MISRII heteromultimers of the disclosure comprise at least one ActRIIA polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452 or amino acids 30-110 of SEQ ID NO: 9. In some embodiments, ActRIIA:MISRII heteromultimers of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID NO: 50, 75, and 79. Preferably, ActRIIA:MISRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ActRIIA and/or MISRII). In other preferred embodiments, ActRIIA:MISRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ActRIIA:MISRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ActRIIA and MISRII homomultimers). ActRIIA:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ActRIIA:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ActRIIB:BMPRII heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists

essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454 or amino acids 29-109 of SEQ ID NO: 1 or 25-131 of SEQ ID NO: 1. In some embodiments, ActRIIB:BMPRII heteromultimers of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 and 71. Preferably, ActRIIB:BMPRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ActRIIB and/or BMPRII). In other preferred embodiments, ActRIIB:BMPRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ActRIIB:BMPRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ActRIIB and BMPRII homomultimers). ActRIIB:BMPRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ActRIIB:BMPRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ActRIIB:TGFBRII heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454 or amino acids 29-109 of SEQ ID NO: 1 or 25-131 of SEQ ID NO: 1. In some embodiments, ActRIIB:TGFBRII

heteromultimers of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, ActRIIB:TGFBRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ActRIIB and/or TGFBRII). In other preferred embodiments, ActRIIB:TGFBRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ActRIIB:TGFBRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ActRIIB and TGFBRII homomultimers). ActRIIB:TGFBRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ActRIIB:TGFBRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, ActRIIB:MISRII heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454 or amino acids 29-109 of SEQ ID NO: 1 or 25-131 of SEQ ID NO: 1. In some embodiments, ActRIIB:MISRII heteromultimers of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458

or amino acids 24-116 of SEQ ID NO: 50, 75, and 79. Preferably, ActRIIB:MISRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of ActRIIB and/or MISRII). In other preferred embodiments, ActRIIB:MISRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, ActRIIB:MISRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, ActRIIB and MISRII homomultimers). ActRIIB:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, ActRIIB:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, BMPRII:TGFBRII heteromultimers of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. In some embodiments, BMPRII:TGFBRII heteromultimers of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. Preferably, BMPRII:TGFBRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of BMPRII and/or TGFBRII). In other preferred embodiments, BMPRII:TGFBRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, BMPRII:TGFBRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, BMPRII and TGFBRII homomultimers). BMPRII:TGFBRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, BMPRII:TGFBRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, BMPRII:MISRII heteromultimers of the disclosure comprise at least one BMPRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456 or amino acids 34-123 of SEQ ID NO: 46 or 71. In some embodiments, BMPRII:MISRII heteromultimers of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID NO: 50, 75, or 79. Preferably, BMPRII:MISRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of BMPRII and/or MISRII). In other preferred embodiments, BMPRII:MISRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC,

activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, BMPRII:MISRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, BMPRII and MISRII homomultimers). BMPRII:MISRII heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, BMPRII:MISRII heteromultimers of the disclosure are heterodimers.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof, and at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. In some embodiments, TGFBRII:MISRII heteromultimers of the disclosure comprise at least one TGFBRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462 or amino acids 44-168 of SEQ ID NO: 67 or amino acids 51-143 of SEQ ID NO: 42. In some embodiments, TGFBRII:MISRII heteromultimers of the disclosure comprise at least one MISRII polypeptide that comprises, consists, or consists essentially of a sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96% 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458 or amino acids 24-116 of SEQ ID NO: 50, 75, or 79. Preferably, TGFBRII:MISRII heteromultimers of the present disclosure are soluble (*e.g.*, comprise an extracellular domain of TGFBII and/or MISRII). In other preferred embodiments, TGFBRII:MISRII heteromultimers 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 (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). In some embodiments, TGFBRII:MISRII heteromultimers of the disclosure have different ligand binding specificities/profiles compared to their corresponding homomultimer complexes (*i.e.*, TGFBRII and MISRII homomultimers). TGFBRII:MISRII

heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further oligomeric structures. In certain preferred embodiments, TGFBRII:MISRII heteromultimers of the disclosure are heterodimers.

In some embodiments, the present disclosure contemplates making functional variants by modifying the structure of a TGF-beta superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) and/or a TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII) for such purposes as enhancing therapeutic efficacy or stability (*e.g.*, shelf-life and resistance to proteolytic degradation *in vivo*). Variants can be produced by amino acid substitution, deletion, addition, or combinations thereof. For instance, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*e.g.*, conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of a polypeptide of the disclosure results in a functional homolog can be readily determined by assessing the ability of the variant polypeptide to produce a response in cells in a fashion similar to the wild-type polypeptide, or to bind to one or more TGF-beta superfamily ligands including, for example, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty.

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

In certain embodiments, the present disclosure contemplates specific mutations of a TGF-beta superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) and/or a TGF-beta superfamily type II receptor polypeptide (*e.g.*,

ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII) receptor of the disclosure so as to alter the glycosylation of the polypeptide. Such mutations may be selected so as to introduce or eliminate one or more glycosylation sites, such as O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine or asparagine-X-serine (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. Removal of one or more carbohydrate moieties present on a polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of a polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* [Meth. Enzymol. (1987) 138:350]. The sequence of a polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect, and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, TGF-beta superfamily type I and II receptor complexes of the present disclosure for use in humans may be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other mammalian expression cell lines are expected to be useful as well.

The present disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of a TGF-beta superfamily type I receptor

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

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

herein and those of common knowledge in the art. A SMAD-responsive reporter gene may be used in such cell lines to monitor effects on downstream signaling.

Combinatorial-derived variants can be generated which have increased selectivity or generally increased potency relative to a reference TGF-beta superfamily heteromultimer 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 intracellular half-lives dramatically different than the corresponding unmodified TGF-beta superfamily 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 within the cell. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter one or more activities of the TGF-beta superfamily heteromultimer complex including, for example, immunogenicity, half-life, and solubility.

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

There are many ways by which the library of potential homologs can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes can then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art. See, *e.g.*, Narang, SA (1983) Tetrahedron 39:3; Itakura *et al.* (1981) Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura *et al.* (1984) Annu. Rev. Biochem. 53:323; Itakura *et al.* (1984) Science 198:1056; Ike *et al.* (1983) Nucleic Acid Res. 11:477. Such techniques have been employed in the directed evolution of other proteins. See, *e.g.*,

Scott *et al.*, (1990) Science 249:386-390; Roberts *et al.* (1992) PNAS USA 89:2429-2433; Devlin *et al.* (1990) Science 249: 404-406; Cwirla *et al.*, (1990) PNAS USA 87: 6378-6382; as well as U.S. Patent Nos: 5,223,409, 5,198,346, and 5,096,815.

Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, TGF-beta superfamily 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 *et al.* (1993) Eur. J. Biochem. 218:597-601; Nagashima *et al.* (1993) J. Biol. Chem. 268:2888-2892; Lowman *et al.* (1991) Biochemistry 30:10832-10838; and Cunningham *et al.* (1989) Science 244:1081-1085], by linker scanning mutagenesis [see, *e.g.*, Gustin *et al.* (1993) Virology 193:653-660; and Brown *et al.* (1992) Mol. Cell Biol. 12:2644-2652; McKnight *et al.* (1982) Science 232:316], by saturation mutagenesis [see, *e.g.*, Meyers *et al.*, (1986) Science 232:613]; by PCR mutagenesis [see, *e.g.*, Leung *et al.* (1989) Method Cell Mol Biol 1:11-19]; or by random mutagenesis, including chemical mutagenesis [*see, e.g.*, Miller *et al.* (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, NY; and Greener *et al.* (1994) Strategies in Mol Biol 7:32-34]. Linker scanning mutagenesis, particularly in a combinatorial setting, is an attractive method for identifying truncated (bioactive) forms of TGF-beta superfamily type I and/or II receptor polypeptides.

A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and, for that matter, for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TGF-beta superfamily 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 relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include TGF-beta superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE,

nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty) binding assays and/or TGF-beta superfamily ligand-mediated cell signaling assays.

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

In certain aspects, the polypeptides disclosed herein may form protein complexes comprising at least one TGF-beta superfamily type I polypeptide associated, covalently or non-covalently, with at least one type II receptor polypeptide. Preferably, polypeptides disclosed herein form 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., Figures 1, 2, and 15-17). In some embodiments, TGF-beta superfamily type I and/or type II receptor polypeptides of the present disclosure comprise at least one multimerization domain. As disclosed herein, the term "multimerization domain" refers to an amino acid or sequence of amino acids that promote covalent or non-covalent interaction between at least a first polypeptide and at least a second polypeptide. Polypeptides disclosed herein may be joined covalently or non-covalently to a multimerization domain. Preferably, a multimerization domain promotes interaction between a first polypeptide (*e.g.*, TGF-beta superfamily type I polypeptide) and a second polypeptide (*e.g.*, TGF-beta superfamily type II polypeptide) to promote heteromultimer formation (*e.g.*, heterodimer formation), and optionally hinders or otherwise

disfavors homomultimer formation (*e.g.*, homodimer formation), thereby increasing the yield of desired heteromultimer (see, e.g., Figure 2).

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

In certain aspects, a multimerization domain may comprise one component of an interaction pair. In some embodiments, the polypeptides disclosed herein may form protein complexes comprising a first polypeptide covalently or non-covalently associated with a second polypeptide, wherein the first polypeptide comprises the amino acid sequence of a TGF-beta superfamily type I polypeptide and the amino acid sequence of a first member of an interaction pair; and the second polypeptide comprises the amino acid sequence of a TGF-beta superfamily type II polypeptide and the amino acid sequence of a second member of an interaction pair. The interaction pair may be any two polypeptide sequences that interact to form a complex, particularly a heterodimeric complex although operative embodiments may also employ an interaction pair that can form a homodimeric complex. One member of the interaction pair may be fused to a TGF-beta superfamily type I or type II polypeptide as described herein, including for example, a polypeptide sequence comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of any one of SEQ ID NOs: 14, 15, 124, 126, 171, 172, 413, 414, 463, 464, 18, 19, 136, 138, 173, 174, 421, 422, 465, 466, 22, 23, 115, 117, 175, 176, 407, 408, 467, 468, 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, 470,

30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, 472, 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, 474, 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, 476, 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, 452, 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, 454, 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, 456, 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, 458, 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462. An interaction pair may be selected to confer an improved property/activity such as increased serum half-life, or to act as an adaptor on to which another moiety is attached to provide an improved property/activity. For example, a polyethylene glycol moiety may be attached to one or both components of an interaction pair to provide an improved property/activity such as improved serum half-life.

The first and second members of the interaction pair may be an asymmetric pair, meaning that the members of the pair preferentially associate with each other rather than self-associate. Accordingly, first and second members of an asymmetric interaction pair may associate to form a heterodimeric complex (see, e.g., 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 complex or a heterodimeric complex. Optionally, the first member of the interaction pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates covalently with the second member of the interaction pair. Optionally, the first member of the interaction pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates non-covalently with the second member of the interaction pair.

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

domain. In other cases, the mutant Fc domain having one or more of these mutations (e.g., Asn-434 mutation) has increased ability of binding to the MHC class I-related Fc-receptor (FcRN) relative to a wildtype Fc domain.

An example of a native amino acid sequence that may be used for the Fc portion of human IgG1 (G1Fc) is shown below (SEQ ID NO: 208). Dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants. In part, the disclosure provides polypeptides comprising, consisting of, or consisting essentially of an amino acid sequence with 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 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).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK        (SEQ ID NO: 208)
```

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 data base conflicts in the sequence (according to UniProt P01859). In part, the disclosure provides polypeptides comprising, consisting of, or consisting essentially of an amino acid sequence with 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 209.

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

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, consisting of, or consisting essentially of an amino acid sequence with 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NOs: 210 and 211.

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

  1  ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
 51  SCDTPPPCPR CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
101  EDPEVQFKWY VDGVEVHNAK TKPREEQYNS TFRVVSVLTV LHQDWLNGKE
151  YKCKVSNKAL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL
201  VKGFYPSDIA VEWESSGQPE NNYNTTPPML DSDGSFFLYS KLTVDKSRWQ
251  QGNIFSCSVM HEALHNRFTQ KSLSLSPGK        (SEQ ID NO: 211)
```

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, consisting of, or consisting essentially of an amino acid sequence with 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 212.

```
  1  ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ
 51  EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE
101  YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL
151  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ
201  EGNVFSCSVM HEALHNHYTQ KSLSLSLGK      (SEQ ID NO: 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_H3 Positions in Different Numbering Systems		
G1Fc (Numbering begins at first threonine in hinge region)	IgG1 heavy chain constant domain (Numbering begins at C_H1)	IgG1 heavy chain (EU numbering scheme of Kabat et al., 1991*)
Y127	Y232	Y349
S132	S237	S354
E134	E239	E356
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 *Sequences of Proteins of Immunological Interest*, 5th ed., Vol. 1, NIH, Bethesda, MD.		

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

Various methods are known in the art that increase desired pairing of Fc-containing fusion polypeptide chains in a single cell line to produce a preferred asymmetric fusion protein at acceptable yields [see, for example, Klein et al (2012) mAbs 4:653-663; and Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Methods to obtain desired pairing of Fc-containing chains include, but are not limited to, charge-based pairing (electrostatic steering), "knobs-into-holes" steric pairing, SEEDbody pairing, and leucine zipper-based pairing. See, for example, Ridgway et al (1996) Protein Eng 9:617-621; Merchant et al (1998) Nat Biotech 16:677-681; Davis et al (2010) Protein Eng Des Sel 23:195-202; Gunasekaran et al (2010); 285:19637-19646; Wranik et al (2012) J Biol Chem 287:43331-43339; US5932448; WO 1993/011162; WO 2009/089004, and WO 2011/034605. As described herein, these methods may be used to generate heterodimers comprising TGF-beta superfamily type I and type II receptor polypeptides, at least two different TGF-beta superfamily type I receptor polypeptides, and at least two different TGF-beta superfamily type II receptor polypeptides. See Figures 15-17.

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

Where a suitably positioned and dimensioned protuberance or cavity exists at the interface of either the first or second polypeptide, it is only necessary to engineer a corresponding cavity or protuberance, respectively, at the adjacent interface.

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

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

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

that can be used, alone or in combination, to enhance heteromultimer formation of the polypeptide complexes disclosed herein.

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

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

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered to be complementary on the basis of charge pairing (electrostatic steering). One of a pair of Fc sequences with electrostatic

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

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

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

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered for steric complementarity. In part, the disclosure provides knobs-into-holes pairing as an example of steric complementarity. One of a pair of Fc sequences with steric complementarity can be arbitrarily fused to the TGF-beta superfamily type I or type II polypeptide of the construct, with or without an optional linker, to generate a TGF-beta superfamily type I or type II fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc to favor generation of the desired multichain construct. In this example based on knobs-into-holes pairing, SEQ ID NO: 202 [human G1Fc(T144Y)] and SEQ ID NO: 203 [human

G1Fc(Y185T)] are examples of complementary Fc sequences in which the engineered amino acid substitutions are double underlined, and the TGF-beta superfamily type I or type II polypeptide of the construct can be fused to either SEQ ID NO: 202 or SEQ ID NO: 203, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 202 and 203).

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

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

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

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

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
```

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

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered to generate interdigitating β-strand segments of human IgG and IgA C_H3 domains. Such methods include the use of strand-exchange engineered domain (SEED) C_H3 heterodimers allowing the formation of SEEDbody fusion proteins [see, for example, Davis et al (2010) Protein Eng Design Sel 23:195-202]. One of a pair of Fc sequences with SEEDbody complementarity can be arbitrarily fused to the TGF-beta superfamily type I or type II polypeptide of the construct, with or without an optional linker, to generate a TGF-beta superfamily type I or type II fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc to favor generation of the desired multichain construct. In this example based on SEEDbody (Sb) pairing, SEQ ID NO: 206 [hG1Fc(Sb_{AG})] and SEQ ID NO: 207 [hG1Fc(Sb_{GA})] are examples of complementary IgG Fc sequences in which the engineered amino acid substitutions from IgA Fc are double underlined, and the TGF-beta superfamily type I or type II polypeptide of the construct can be fused to either SEQ ID NO: 206 or SEQ ID NO: 207, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG1Fc, hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate an Fc monomer which may be used in the complementary IgG-IgA pair below (SEQ ID NOs: 206 and 207).

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

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

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains with a cleavable leucine zipper domain attached at the C-terminus of the

Fc C_H3 domains. Attachment of a leucine zipper is sufficient to cause preferential assembly of heterodimeric antibody heavy chains. See, e.g., Wranik et al (2012) J Biol Chem 287:43331-43339. As disclosed herein, one of a pair of Fc sequences attached to a leucine zipper-forming strand can be arbitrarily fused to the TGF-beta superfamily type I or type II polypeptide of the construct, with or without an optional linker, to generate a TGF-beta superfamily type I or type II fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence attached to a complementary leucine zipper-forming strand to favor generation of the desired multichain construct. Proteolytic digestion of the construct with the bacterial endoproteinase Lys-C post purification can release the leucine zipper domain, resulting in an Fc construct whose structure is identical to that of native Fc. In this example based on leucine zipper pairing, SEQ ID NO: 213 [hG1Fc-Ap1 (acidic)] and SEQ ID NO: 214 [hG1Fc-Bp1 (basic)] are examples of complementary IgG Fc sequences in which the engineered complimentary leucine zipper sequences are underlined, and the TGF-beta superfamily type I or type II polypeptide of the construct can be fused to either SEQ ID NO: 213 or SEQ ID NO: 214, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that leucine zipper-forming sequences attached, with or without an optional linker, to hG1Fc, hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate an Fc monomer which may be used in the complementary leucine zipper-forming pair below (SEQ ID NOs: 213 and 214).

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

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

As described above, 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 [Klein et al (2012) mAbs 4:653-663; and Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. In addition, heteromultimers as described herein may be generated using a combination of heavy and light chain fusion proteins comprising either an TGF-beta superfamily type I or TGF-beta superfamily type II polypeptide. For example, in some embodiments, a TGF-beta superfamily type I receptor polypeptide polypeptide may be fused, with or without a linker domain, to an immunoglobulin heavy chain (IgG1, IgG2, IgG3, IgG4, IgM, IgA1, or IgA2) that comprises at least a portion of the C_H1 domain. Similarly, a TGF-beta superfamily type II receptor polypeptide may be fused, with or without a linker domain, to an immunoglobulin light chain (kappa or lambda) that comprises at least a portion of the light chain constant domain (C_L). In alternative embodiments, a TGF-beta superfamily type I receptor polypeptide may be fused, with or without a linker domain, to an immunoglobulin heavy chain (IgG1, IgG2, IgG3, IgG4, IgM, IgA1, or IgA2) that comprises at least a portion of the C_H1 domain, and a TGF-beta superfamily type II receptor polypeptide may be fused, with or without a linker domain, to an immunoglobulin light chain (kappa or lambda) that comprises at least a portion of the light chain constant domain (C_L). This design takes advantage of the natural ability of the heavy chains to heterodimerize with light chains. In particular, heterodimerization of a heavy and light chain occurs between the C_H1 with the C_L, which is generally stabilized by covalent linking of the two domains via a disulfide bridge. Constructs employing the full-length heavy chain, or at least a portion of the heavy chain comprising the hinge region, could give rise to antibody-like molecules comprising two "light chains" and two "heavy chains". See Figure 16. A potential advantage of this design is that it may more closely mimic the naturally occurring TGF-beta superfamily type I receptor polypeptide-ligand-TGF-beta superfamily type II receptor polypeptide complex and may display higher affinity for the ligand than comparable single heterodimers. In some embodiments, this design may be modified by incorporating various heavy chain truncations including, for example, truncations that comprise the C_H1 domain and some or all of the hinge domain (giving rise to $F(ab')_2$-like molecules) as well as truncations that only comprise the C_H1 domain or a fragment thereof (giving rise to Fab-like molecules). See Figure 16G. Various methods for designing such heteromultimer constructs are described in US 2009/0010879, Klein et al [(2012) mAbs 4:653-663], and Spiess et al [(2015) Molecular Immunology 67(2A): 95-106] the contents of which are incorporated in their entirety herein.

In some embodiments, it is desirable to generate antibody-like heterodimers comprising at least one branch of the complex comprising an TGF-beta superfamily type I receptor polypeptide-C_L:TGF-beta superfamily type II receptor polypeptide-C_H1 heterodimer pair and at least a second branch comprising an TGF-beta superfamily type II receptor polypeptide-C_L:TGF-beta superfamily type I receptor polypeptide r-C_H1 heterodimer pair. See, e.g., Figure 16B. Such heterodimer complexes can be generated, for example, using combinations of heavy chain and light chain asymmetrical pairing technologies [Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. For example, in CrossMab technology, [Schaefer et al (2011). Proc. Natl. Acad. Sci. U.S.A. 108: 11187–11192] light chain mispairing is overcome using domain crossovers and heavy chains heterodimerized using knobs-into-holes [Merchant et al (1998) Nat. Biotechnol. 16: 677–681]. For the domain crossovers either the variable domains or the constant domains are swapped between light and heavy chains to create two asymmetric Fab arms that drive cognate light chain pairing while preserving the structural and functional integrity of the variable domain [Fenn et al (2013) PLoS ONE 8: e61953]. An alternative approach for overcoming light chain mispairing is designing heavy and light chains with orthogonal Fab inter-faces [Lewis (2014) Nat. Biotechnol. 32: 191–198]. This has been accomplished by computational modeling [Das et al (2008) Annu. Rev. Biochem.77: 363–382] in combination with X-ray crystallography to identify mutations at the V_H/V_L and C_H1/C_L interfaces. For the heterodimers generated using this methodology, it may be necessary to engineer mutations into both V_H/V_L and C_H1/C_L interfaces to minimize heavy/light chain mispairing. The designed orthogonal Fab interface may be used in conjunction with a heavy chain heterodimerization strategy to facilitate efficient IgG production in a single host cell. Electrostatic steering may also be used to generate orthogonal Fab interfaces to facilitate the construction of such heterodimers. Peptide linkers may be used to ensure cognate pairing of light and heavy chains in a format known as "LUZ-Y" [Wranik et al (2012) J. Biol. Chem. 287: 43331-43339], wherein heavy chain heterodimerization is accomplished using leucine zippers which may be subsequently removed by proteolysis in vitro.

Alternatively, heteromultimers may comprise one or more single-chain ligand traps as described herein, optionally which may be covalently or non-covalently associated with one or more TGF-beta superfamily type I receptor polypeptides or TGF-beta superfamily type I receptor polypeptides as well as additional TGF-beta superfamily type I receptor polypeptide:TGF-beta superfamily type II receptor polypeptide single chain ligand traps [US

2011/0236309 and US2009/0010879]. See Figure 12. As described herein, single-chain ligand traps do not require fusion to any multimerization domain such as coiled-coil Fc domains to be multivalent. In general, single-chain ligand traps of the present disclosure comprise at least one TGF-beta superfamily type I receptor polypeptide domain and one TGF-beta superfamily type I receptor polypeptide domain. The TGF-beta superfamily type I receptor polypeptide and TGF-beta superfamily type I receptor polypeptide domains, generally referred to herein as binding domains (BD), optionally may be joined by a linker region.

For example, in one aspect, the present disclosure provides heteromultimers comprising a polypeptide having the following structure:

$$(<BD1>\text{-linker1})_k\text{-}[<BD2>\text{-linker2-}\{<BD3>\text{-linker3}\}_f]_n\text{-}(<BD4>)_m\text{-}(\text{linker4-}BD5>_d)_h$$

where: n and h are independently greater than or equal to one; d, f, m, and k are independently equal to or greater than zero; BD1, BD2, BD3, BD4, and BD5 are independently TGF-beta superfamily type I receptor polypeptide or TGF-beta superfamily type II receptor polypeptide domains, wherein at least one of BD1, BD2, BD3, and BD4 is an TGF-beta superfamily type I receptor polypeptide domain, and wherein at least one of BD1, BD2, BD3, and BD4 is an TGF-beta superfamily type II receptor polypeptide domain, and linker1, linker2, linker3, and linker 4 are independently greater than or equal to zero. In some embodiment, TGF-beta superfamily type I receptor polypeptide:TGF-beta superfamily type II receptor polypeptide single-chain traps comprise at least two different TGF-beta superfamily type I receptor polypeptide. In some embodiments, TGF-beta superfamily type I receptor polypeptide:TGF-beta superfamily type II receptor polypeptide single-chain traps comprise at least two different TGF-beta superfamily type II receptor polypeptide polypeptides. In some embodiment, TGF-beta superfamily type I receptor polypeptide:TGF-beta superfamily type I receptor polypeptide single-chain traps comprise at least two different linkers. Depending on the values of selected for d, f, h, k, m, and n, the heteromultimer structure may comprise a large number of repeating units in various combinations or may be a relatively simple structure.

In another aspect, the present disclosure provides heteromultimers comprising a polypeptide having the following structure:

<BD1>-linker1-<BD2>

In yet another aspect, the present disclosure provides heteromultimers comprising a polypeptide having the following structure:

<BD1>-(linker2-<BD2>)$_n$

where n is greater than or equal one.

Another aspect of the invention provides heteromultimers comprising a polypeptide having the following structure:

(<BD1>-linker1-<BD1>)$_f$-linker2-(<BD2>-linker3-<BD3>)$_g$

wherein f and g are greater than or equal to one.

In an embodiment where BD2 and BD3 are the same, and f and g are the same number, this can result in a substantially mirror symmetric structure around linker 2, subject to differences in the linkers. In instances where BD2 is different from BD3 and/or where f and g are different numbers, different structures will be produced. It is within the capacity of one of ordinary skill in the art to select suitable binding domains, linkers, and repeat frequencies in light of the disclosure herein and knowledge in the art. Specific, non-limiting examples of such single-chain ligand traps in accordance with the present disclosure are represented schematically in Figure 18.

The linkers (1, 2, 3, and 4) may be the same or different. The linker region provides a segment that is distinct from the structured ligand-binding domains of TGF-beta superfamily type I receptor polypeptide and TGF-beta superfamily type II receptor polypeptide and thus can be used for conjugation to accessory molecules (e.g., molecules useful in increasing stability such as PEGylation moieties) without having to chemically modify the binding domains. The linker may include an unstructured amino acid sequence that may be either the same as or derived from conservative modifications to the sequence of a natural unstructured region in the extracellular portion of the receptor for the ligand of interest or another receptor in the TGF-β superfamily. In other instances, such linkers may be entirely artificial in composition and origin but will contain amino acids selected to provide an unstructured flexible linker with a low likelihood of encountering electrostatic or steric hindrance complications when brought into close proximity to the ligand of interest. Linker length will be considered acceptable when it permits binding domains located on each of the N- and C-termini of the linker to bind their natural binding sites on their natural ligand such that, with both binding domains so bound, the ligand is bound with a higher affinity than it would be bound by binding of only one of the binding domains. In some instances, the number of

amino acid residues in the linker of either natural or artificial origin is selected to be equal to or greater than the minimum required distance for simultaneous (bridged) binding to two binding sites on the TGF-beta superfamily type I receptor polypeptide and/or TGF-beta superfamily type II receptor polypeptide ligand. For example, and without wishing to be limiting in any manner, the linker length may be between about 1- 10 amino acids, 10-20 amino acids, 18-80 amino acids, 25-60 amino acids, 35-45 amino acids, or any other suitable length.

Linkers may be designed to facilitate purification of the polypeptide. The exact purification scheme chosen will determine what modifications are needed, for example and without wishing to be limiting, additions of purification "tags" such as His tags is contemplated; in other examples, the linker may include regions to facilitate the addition of cargo or accessory molecules. When such additions affect the unstructured nature of the linker or introduce potential electrostatic or steric concerns, appropriate increases to the linker length will be made to ensure that the two binding domains are able to bind their respective sites on the ligand. In light of the methods and teachings herein, such determinations could be made routinely by one skilled in the art.

In addition, the present design permits linkage of other cargo molecules (for example imaging agents like fluorescent molecules), toxins, etc. For example, and without wishing to be limiting in any manner, single-chain polypeptides can be modified to add one or more cargo and/or accessory molecules (referred to collectively herein by R1, R2, R3, R4, etc.):

R3 R4 R5 R6 R7 R8 R9

R1 – (<BD1>-linker1)$_k$-[<BD2>-linker2-{<BD3>-linker3}$_f$]$_n$-(<BD4>)$_m$-(linker4-BD5>$_d$)$_h$ – R2

Without limiting the generality of R substituents available, R1, R2, R3, R4, R5, R6, R7, R8, R9, may or may not be present; when present, they may be the same or different, and may independently be one or more of: a fusion protein for targeting, for example, but not limited to such as an antibody fragment (e.g. single chain Fv) and/or a single domain antibody (sdAb); a radiotherapy and/or imaging agent, for example, but not limited to a radionuceotide (e.g. ^{123}I, ^{111}In, ^{18}F, ^{64}C, ^{68}Y, ^{124}I, ^{131}I, ^{90}Y, ^{177}Lu, ^{57}Cu, ^{213}Bi, ^{211}At), a fluorescent dye (e.g. Alexa Fluor, Cy dye) and/or a fluorescent protein tag (e.g. GFP, DsRed); a cytotoxic agent for chemotherapy, for example, but not limited to doxorubicin, calicheamicin, a maytansinoid derivatives (e.g. DM1, DM4), a toxin (eg. truncated

Pseudomonas endotoxin A, diphteria toxin); a nanoparticle-based carrier, for example, but not limited to polyethylene glycol (PEG), a polymer-conjugated to drug, nanocarrier or imaging agent (e.g. of a polymer N-(2-hydorxylpropyl) methacrylamide (HPMA), glutamic acid, PEG, dextran); a drug (for example, but not limited to doxorubicin, camptothecin, paclitaxel, palatinate); a nanocarrier, for example, but not limited to a nanoshell or liposome; an imaging agent, for example, but not limited to Supermagnetic Iron Oxide (SPIO); a dendrimer; and/or a solid support for use in ligand purification, concentration or sequestration (e.g. nanoparticles, inert resins, suitable silica supports).

In general, it will not be preferable to have cargo or accessory molecules in all possible positions, as this may cause steric or electrostatic complications. However, the effects of adding a cargo or accessory molecule to any given position or positions on the structure can be determined routinely in light of the disclosure herein by modeling the linker between the binding domains and carrying out molecular dynamics simulations to substantially minimize molecular mechanics energy and reduce steric and electrostatic incompatibility between the linker and the TGF-beta superfamily type I receptor polypeptide and TGF-beta superfamily type II receptor polypeptideas taught herein.

It may be preferable to add the cargo or accessory molecule to the linker portion of the agent, rather to the binding domain, to reduce the likelihood of interference in binding function. However, addition to the binding domain is possible and could be desirable in some instances and the effect of such an addition can be determined routinely in advance by modeling the binding agent and the linker with the proposed addition as described herein.

Conjugation methodologies may be performed using commercial kits that enable conjugation via common reactive groups such as primary amines, succinimidyl (NHS) esters and sulfhydral-reactive groups. Some non-limiting examples are: Alexa Fluor 488 protein labeling kit (Molecular Probes, Invitrogen detection technologies) and PEGylation kits (Pierce Biotechnology Inc.).

In certain aspects, TGF-beta superfamily type I receptor polypeptide:TGF-beta superfamily type II receptor polypeptide single-chain traps may be covalently or non-covalently associated with one or more TGF-beta superfamily type I receptor polypeptides or TGF-beta superfamily type II receptor polypeptide as well as additional TGF-beta superfamily type I receptor polypeptide:TGF-beta superfamily type II receptor polypeptide single chain ligand traps to form higher order heteromultimers, which may be used in

accordance with the methods described herein. See, e.g., Figure 19. For example, an TGF-beta superfamily type I receptor polypeptide: TGF-beta superfamily type II receptor polypeptide single chain ligand trap may further comprise a multimerization domain as described herein. In some embodiments, TGF-beta superfamily type I receptor polypeptide:TGF-beta superfamily type II receptor polypeptide single chain ligand traps comprise a constant domain of an Ig immunoglobulin. Such immunoglobulins constant domains may be selected to promote symmetrical or asymmetrical complexes comprising at least one single-chain TGF-beta superfamily type I receptor polypeptide:TGF-beta superfamily type II receptor polypeptide trap.

In certain aspects, an TGF-beta superfamily type I receptor polypeptide: TGF-beta superfamily type II receptor polypeptide single-chain trap, or combinations of such traps, may be used as TGF-beta superfamily antagonists to treat or prevent an TGF-beta superfamily disorder or disease as described herein (e.g., kidney disease and/or a metabolic disorder or condition).

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

For example, a TGF-beta superfamily type I and/or type II receptor fusion protein may comprise an amino acid sequence as set forth in the formula A-B-C. The B portion corresponds to a TGF-beta superfamily type I and/or type II receptor polypeptide domain. The A and C portions may be independently zero, one, or more than one amino acid, and both the A and C portions when present are heterologous to B. The A and/or C portions may be attached to the B portion via a linker sequence. A linker may be rich in glycine (*e.g.*, 2-10, 2-5, 2-4, 2-3 glycine residues) or glycine and proline residues and may, for example, contain a single sequence of threonine/serine and glycines or repeating sequences of threonine/serine and/or glycines, *e.g.*, GGG (SEQ ID NO: 58), GGGG (SEQ ID NO: 59), TGGGG (SEQ ID NO: 60), SGGGG (SEQ ID NO: 61), TGGG (SEQ ID NO: 62), or SGGG (SEQ ID NO: 63)

singlets, or repeats. In certain embodiments, a TGF-beta superfamily type I and/or type II receptor fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a leader (signal) sequence, B consists of a TGF-beta superfamily type I and/or type II receptor polypeptide domain, and C is a polypeptide portion that enhances one or more of *in vivo* stability, *in vivo* half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. In certain embodiments, a TGF-beta superfamily type I and/or type II receptor fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a TPA leader sequence, B consists of a TGF-beta superfamily type I and/or type II receptor polypeptide domain, and C is an immunoglobulin Fc domain. Preferred fusion proteins comprise the amino acid sequence set forth in any one of SEQ ID NOs: 100, 102, 104, 106, 112, 114, 115, 117, 118, 120, 121, 123, 124, 126, 127, 129, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, and 416.

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

In certain embodiments, TGF-beta superfamily type I and/or type II receptor polypeptides of the present disclosure 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 proteins (including, for example, fusion proteins comprising a TGF-beta superfamily type I and/or type II receptor polypeptide domain and a stabilizer domain), modifications of a glycosylation site (including, for example, addition of a glycosylation site to a polypeptide of the disclosure), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from a polypeptide of the disclosure). As used herein, the term "stabilizer domain" not only refers to a fusion domain (*e.g.*, an immunoglobulin Fc domain) as in the case of fusion proteins, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous moiety, such as polyethylene glycol.

In preferred embodiments, TGF-beta superfamily 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 heteromultimer complex of the disclosure is purified to greater than 95%, 96%, 97%, 98%, or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). Methods for assessment of antibody purity are well known in the art [See, *e.g.*, Flatman *et al.*, (2007) J. Chromatogr. B 848:79-87]. In some embodiments, heteromultimer preparations of the disclosure are substantially free of TGF-beta superfamily type I receptor polypeptide homomultimers and/or TGF-beta superfamily type II receptor polypeptide homomultimers. For example, in some embodiments, heteromultimer preparations comprise less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% of TGF-beta superfamily type I receptor polypeptide homomultimers. In some embodiments, heteromultimer preparations comprise less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% of TGF-beta superfamily type II receptor polypeptide homomultimers. In some embodiments, heteromultimer preparations comprise less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% of TGF-beta superfamily type I

receptor polypeptide homomultimers and less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2%, or less than 1% of TGF-beta superfamily type II receptor polypeptide homomultimers.

In certain embodiments, TGFβ superfamily type I and/or type II receptor polypeptides, as well as 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 TGFβ superfamily type I and/or type II receptor polypeptides by using, for example, a protease, *e.g.*, trypsin, thermolysin, chymotrypsin, pepsin, or paired basic amino acid converting enzyme (PACE). Computer analysis (using a commercially available software, *e.g.*, MacVector, Omega, PCGene, Molecular Simulation, Inc.) can be used to identify proteolytic cleavage sites.

With respect to antibodies that bind to and antagonize ligands that bind to TGF-beta type I receptor polypeptide:TGF-beta type II receptor polypeptide heteromultimers of the disclosure (e.g., BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty) it is contemplated that an antibody may be designed as a bispecific antibody comprising a first portion that binds to an epitope of such ligand, such that the first portion of the antibody competes for binding with a type I receptor and comprising a second portion that binds to an epitope of such ligand, such that the second portion of the antibody competes for binding with a type II receptor. In this manner, a bispecific antibody targeting a single ligand can be designed to mimic the dual type I-type II receptor binding blockade that may be conferred by an ALK7:ActRIIB heteromultimer. Similarly it is contemplated that the same effect could be

achieved using a combination of two or more antibodies wherein at least a first antibody binds to an epitope of such ligand, such that the first antibody competes for binding with a type I receptor and at least a second antibody binds to an epitope of such ligand, such that the second antibody competes for binding with a type II receptor.

5

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

In certain embodiments, the present disclosure provides isolated and/or recombinant nucleic acids encoding TGFβ superfamily type I and/or type II receptors (including fragments, 10 functional variants, and fusion proteins thereof) disclosed herein. For example, SEQ ID NO: 12 encodes the naturally occurring human ActRIIA precursor polypeptide, while SEQ ID NO: 13 encodes the mature, extracellular domain of ActRIIA. The subject nucleic acids may be single-stranded or double stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making TGF-beta superfamily 15 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 20 different from its natural chromosomal location.

In certain embodiments, nucleic acids encoding TGFβ superfamily type I and/or type II receptor polypeptides of the present disclosure are understood to include nucleic acids that are variants of any one of SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 25 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions, or deletions including allelic variants, and therefore, will include coding sequences that differ from the nucleotide sequence designated in any one of SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 30 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143.

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

In other embodiments, nucleic acids of the present disclosure also include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequence designated in SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143, the complement sequence of SEQ ID NOs: 7, 8, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 69, 70, 73, 74, 77, 78, 81, 82, 85, 86, 89, 90, 93, 94, 303, 304, 307, 308, 311, 312, 101, 105, 113, 116, 119, 122, 125, 128, 131, 134, 137, 140, and 143, or fragments thereof. One of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45 °C, followed by a wash of 2.0 x SSC at 50 °C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50 °C to a high stringency of about 0.2 x SSC at 50 °C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22 °C, to high stringency conditions at about 65 °C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In one embodiment, the disclosure provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a wash at 2 x SSC at room temperature.

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

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

In certain aspects of the present disclosure, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a TGFβ superfamily type I and/or type II receptor polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the TGFβ

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

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

Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells.

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

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

This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence for one or more of the subject TGFβ superfamily type I and/or type II receptor polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a TGFβ superfamily type I and/or type II receptor polypeptide of the disclosure may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells [*e.g.* a Chinese hamster ovary (CHO) cell line]. Other suitable host cells are known to those skilled in the art.

Accordingly, the present disclosure further pertains to methods of producing the subject TGFβ superfamily type I and/or type II receptor polypeptides. For example, a host cell transfected with an expression vector encoding a TGFβ superfamily type I and/or type II receptor polypeptide can be cultured under appropriate conditions to allow expression of the

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

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

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

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

4. Screening Assays

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

There are numerous approaches to screening for therapeutic agents for modulating tissue growth by targeting TGFβ superfamily ligand signaling (*e.g.*, SMAD 2/3 and/or SMAD 1/5/8 signaling). In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb TGFβ superfamily receptor-mediated effects on a selected cell line. In certain embodiments, the assay is carried out to screen and identify compounds that specifically inhibit or reduce binding of a TGF-beta superfamily heteromultimer complex to its binding partner, such as a TGFβ superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). Alternatively, the assay can be used to identify compounds that enhance binding of a TGF-beta superfamily

heteromultimer complex to its binding partner such as a TGFβ superfamily ligand. In a further embodiment, the compounds can be identified by their ability to interact with a TGF-beta superfamily heteromultimer 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, digoxygenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S-transferase (GST), photoactivatible crosslinkers or any combinations thereof.

In many drug-screening programs which test libraries of compounds and natural extracts, high-throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity or bioavailability of the test compound can be

generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity between a TGF-beta superfamily heteromultimer complex and its binding partner (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty).

Merely to illustrate, in an exemplary screening assay of the present disclosure, the compound of interest is contacted with an isolated and purified TGF-beta superfamily heteromultimer 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 heteromultimer complex is then added to a composition containing the appropriate TGF-beta superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). Detection and quantification of heteromultimer-superfamily ligand complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the TGF-beta superfamily 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 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 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 ^{3}H), fluorescently labeled (*e.g.*, FITC), or enzymatically labeled TGF-beta superfamily heteromultimer complex and/or its binding protein, by immunoassay, or by chromatographic detection.

In certain embodiments, the present disclosure contemplates the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between a TGF-beta superfamily heteromultimer 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 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 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 heteromultimer complex of the disclosure. The interaction between the compound and the TGF-beta superfamily 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 heteromultimer complex. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding a TGF-beta superfamily 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 heteromultimer complex, or combinations of TGF-beta superfamily 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 TGFβ superfamily receptor (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, ActRIIB, BMPRII, TGFBRII, and MISRII) and/or a TGFβ superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty). These diseases, disorders or conditions are generally referred to herein as "TGFβ superfamily-associated conditions" or "TGFβ superfamily-associated disorders." In certain embodiments, the present invention provides methods of treating or preventing an individual in need thereof through administering to the individual a therapeutically effective amount of a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily heteromultimer complexes, as described herein. Any of the TGF-beta superfamily heteromultimer complexes of the present disclosure can potentially be employed individually or in combination for therapeutic uses disclosed herein. These methods are particularly aimed at therapeutic and prophylactic treatments of mammals including, for example, rodents, primates, and humans.

As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample. The term "treating" as used herein includes amelioration or elimination of the condition once it has been established. In either case, prevention or treatment may be

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

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

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

In certain embodiments, a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily 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 heteromultimer complexes include: Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), Emery-Dreifuss muscular dystrophy (EDMD), limb-girdle muscular dystrophy (LGMD), facioscapulohumeral muscular dystrophy (FSH or FSHD) (also known as Landouzy-Dejerine), myotonic dystrophy (MMD; also known as Steinert's Disease), oculopharyngeal muscular dystrophy (OPMD), distal muscular dystrophy (DD), congenital muscular dystrophy (CMD).

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

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

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

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

In certain embodiments, a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily 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 heteromultimer complexes may be useful in patients who are diagnosed with subclinical low bone density, as a protective measure against the development of osteoporosis.

In some embodiments, a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily heteromultimer complexes, of the present disclosure may find medical utility in the healing of bone fractures and cartilage defects in humans and other animals. The subject methods and compositions may also have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent is useful for repair of craniofacial defects that are congenital, trauma-induced, or caused by oncologic resection, and is also useful in cosmetic plastic surgery. Further, methods and compositions of the invention may be used in the treatment of periodontal disease and in other tooth repair processes. In certain cases, a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily 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 heteromultimer complexes of the disclosure may also be useful in the treatment of osteoporosis. Further, TGF-beta superfamily heteromultimer complexes may be used in repair of cartilage defects and prevention/reversal of osteoarthritis. Examples of heteromeric complexes useful for inducing bone formation, preventing bone loss, increasing bone mineralization, preventing the demineralization of bone, and/or increasing bone density as described above are ActRIIB-Fc:ALK3-Fc and ActRIIB-Fc:ALK4-Fc.

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 heteromultimer complex or with combinations of TGF-beta superfamily 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 heteromultimer complexes of the disclosure in admixture with a pharmaceutically acceptable vehicle, carrier, or matrix.

In some embodiments, a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily 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 produceds by the body results in osteoporosis and fractures. The most common medications associated with secondary osteoporosis are the corticosteroids, a class of drugs that act like cortisol, a hormone produced naturally by the adrenal glands. Although adequate levels of thyroid hormones are needed for the development of the skeleton, excess thyroid hormone can decrease bone mass over time. Antacids that contain aluminum can lead to bone loss when taken in high doses by people with kidney problems, particularly those undergoing dialysis. Other medications that can cause secondary osteoporosis include phenytoin (Dilantin) and barbiturates that are used to prevent seizures; methotrexate (Rheumatrex, Immunex, Folex PFS), a drug for some forms of arthritis, cancer, and immune disorders; cyclosporine (Sandimmune, Neoral), a drug used to treat some autoimmune diseases and to suppress the immune system in organ transplant patients; luteinizing hormone-releasing hormone agonists (Lupron, Zoladex), used to treat prostate cancer and endometriosis; heparin (Calciparine, Liquaemin), an anticlotting medication; and cholestyramine (Questran) and colestipol (Colestid), used to treat high cholesterol. Bone loss resulting from cancer therapy is widely recognized and termed cancer therapy-induced bone loss (CTIBL). Bone metastases can create cavities in the bone that may be corrected by treatment with a TGF-beta superfamily heteromultimer 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 heteromultimer complex, or combinations of TGF-beta superfamily 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 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 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

heteromultimer complex, or combinations of TGF-beta superfamily heteromultimer complexes, in a patient.

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

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

A patient may also benefit from conjointly receiving a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily 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 commonly in the form of a pill or skin patch that delivers a low dose of approximately 0.3 mg daily or a standard dose of approximately 0.625 mg daily and is effective even when started after age 70. When estrogen is taken alone, it can increase a woman's risk of developing cancer of the uterine lining (endometrial cancer). To eliminate this risk, healthcare providers prescribe the hormone progestin in combination with estrogen (hormone replacement therapy or HT) for those women who have an intact uterus. ET/HT relieves menopause symptoms and has been shown to have a beneficial effect on bone health. Side effects may include vaginal bleeding, breast tenderness, mood disturbances and gallbladder disease. Raloxifene, 60 mg a day, is approved for the prevention and treatment of postmenopausal osteoporosis. It is from a class of drugs called Selective Estrogen Receptor Modulators (SERMs) that have been developed to provide the beneficial effects of estrogens without their potential disadvantages. Raloxifene increases bone mass and reduces the risk of spine fractures. Data are not yet available to demonstrate that raloxifene can reduce the risk of hip and other non-spine fractures. Teriparatide, a form of parathyroid hormone, is approved for the treatment of osteoporosis in postmenopausal women and men who are at high risk for a fracture. This medication stimulates new bone formation and significantly increases bone mineral density. In postmenopausal women, fracture reduction was noted in the spine, hip, foot, ribs and wrist. In men, fracture reduction was noted in the spine, but there were insufficient data to evaluate fracture reduction at other sites. Teriparatide is self-administered as a daily injection for up to 24 months.

In other embodiments, a TGF-beta superfamily heteromultimer complex, or combinations of TGF-beta superfamily 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 heteromultimer complex, or combinations of TGF-beta superfamily heteromultimer complexese, of the disclosure.

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

In certain aspects, a TGF-beta superfamily heteromultimer complex, or a combination of TGF-beta superfamily 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 heteromultimer complex, or a combination of TGF-beta superfamily 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 heteromultimer complex, or a combination of TGF-beta superfamily 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 heteromultimer complex, or a combination of TGF-beta superfamily 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 heteromultimer complex, or a combination of TGF-beta superfamily 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 heteromultimer complex, or a combination of TGF-beta superfamily 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 heteromultimer complex, or a combination of TGF-beta superfamily 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 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 erythematosis (SLE), acute or chronic skin diseases (*e.g.*, psoriasis), inflammatory bowel disease (*e.g.*, Crohn's disease and ulcerative colitis); acute or chronic renal disease or failure, including idiopathic or congenital conditions; acute or chronic liver disease; acute or chronic bleeding;

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

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

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

Anemia resulting from acute blood loss of sufficient volume, such as from trauma or postpartum hemorrhage, is known as acute post-hemorrhagic anemia. Acute blood loss initially causes hypovolemia without anemia since there is proportional depletion of RBCs along with other blood constituents. However, hypovolemia will rapidly trigger physiologic mechanisms that shift fluid from the extravascular to the vascular compartment, which results

in hemodilution and anemia. If chronic, blood loss gradually depletes body iron stores and eventually leads to iron deficiency. In some embodiments, one or more TGF-beta superfamily 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 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 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 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-beta superfamily 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 heteromultimer complexes of the disclosure and one or more additional therapeutic agents for treating MDS including, for example, thalidomide, lenalidomide, azacitadine, decitabine, erythropoietins, deferoxamine, antithymocyte globulin, and filgrastrim (G-CSF).

Originally distinguished from aplastic anemia, hemorrhage, or peripheral hemolysis on the basis of ferrokinetic studies [see, *e.g.*, Ricketts *et al.* (1978) Clin Nucl Med 3:159-164], ineffective erythropoiesis describes a diverse group of anemias in which production of mature RBCs is less than would be expected given the number of erythroid precursors (erythroblasts) present in the bone marrow [Tanno *et al.* (2010) Adv Hematol 2010:358283]. In such anemias, tissue hypoxia persists despite elevated erythropoietin levels due to ineffective production of mature RBCs. A vicious cycle eventually develops in which elevated erythropoietin levels drive massive expansion of erythroblasts, potentially leading to splenomegaly (spleen enlargement) due to extramedullary erythropoiesis [see, *e.g.*, Aizawa *et al.* (2003) Am J Hematol 74:68-72], erythroblast-induced bone pathology [see, *e.g.*, Di Matteo *et al.* (2008) J Biol Regul Homeost Agents 22:211-216], and tissue iron overload, even in the absence of therapeutic RBC transfusions [see, *e.g.*, Pippard *et al.* (1979) Lancet 2:819-821]. Thus, by boosting erythropoietic effectiveness, one or more TGF-beta superfamily heteromultimer complexes of the present disclosure may break the aforementioned cycle and thus alleviate not only the underlying anemia but also the associated complications of elevated erythropoietin levels, splenomegaly, bone pathology, and tissue iron overload. In some embodiments, one or more TGF-beta superfamily 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 heteromultimer complexes of the disclosure, optionally combined with an EPO receptor activator, can be used to treat or prevent a thalassemia syndrome.

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

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

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

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

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

In certain embodiments, the present disclosure provides methods of treating or preventing anemia in an individual in need thereof by administering to the individual a

therapeutically effective amount of one or more TGF-beta superfamily heteromultimer complexes of the disclosure and a EPO receptor activator. In certain embodiments, one or more TGF-beta superfamily 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 of EPO. These methods may be used for therapeutic and prophylactic treatments of a patient.

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

Provided that TGF-beta superfamily heteromultimer complexes of the present disclosure act by a different mechanism than EPO, these antagonists may be useful for increasing red blood cell and hemoglobin levels in patients that do not respond well to EPO. For example, a TGF-beta superfamily heteromultimer 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 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 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 heteromultimer complexes of the disclosure, then onset of administration of the one or more TGF-beta superfamily 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 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 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 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 heteromultimer complexes of the disclosure. Alternatively, a therapeutic regimen may be developed for the patient that combines one or more TGF-beta superfamily 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 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 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 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 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 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 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 heteromultimer complexes of the disclosure or additional dosing with another therapeutic agent. For example, if administration of one or more TGF-beta superfamily heteromultimer complexes of the disclosure of the disclosure results in an increase in blood pressure, red blood cell level, or hemoglobin level, or a reduction in iron stores, then the dose of the one or more TGF-beta superfamily 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 heteromultimer complexes of the disclosure on the one or more hematologic parameters. If administration of one or more TGF-beta superfamily 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 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 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 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 heteromultimer complexes of the disclosure has elevated blood pressure, then dosing with the one or more TGF-beta superfamily 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 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 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 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 more agents of the disclosure in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use. Injectable solutions or dispersions may contain antioxidants, buffers, bacteriostats, suspending agents, thickening agents, or solutes which render the formulation isotonic with the blood of the intended recipient. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical formulations of the present disclosure include water, ethanol, polyols (*e.g.*, glycerol, propylene glycol, polyethylene glycol, *etc.*), vegetable oils (*e.g.*, olive oil), injectable organic esters (*e.g.*, ethyl oleate), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by the use of coating materials (*e.g.*, lecithin), by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

In some embodiments, a therapeutic method of the present disclosure includes administering the pharmaceutical composition systemically, or locally, from an implant or

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

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

In certain embodiments, pharmaceutical compositions of present disclosure can be administered topically. "Topical application" or "topically" means contact of the pharmaceutical composition with body surfaces including, for example, the skin, wound sites, and mucous membranes. The topical pharmaceutical compositions can have various application forms and typically comprises a drug-containing layer, which is adapted to be placed near to or in direct contact with the tissue upon topically administering the composition. Pharmaceutical compositions suitable for topical administration may comprise one or more one or more TGFβ superfamily type I and/or type II receptor polypeptide 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, guars, carrageenans, hyaluronic acids, inulin, starches, modified starches, agarose, methylcelluloses, plant gums, hylaronans, hydrogels, gelatins, glycosaminoglycans, carboxymethyl celluloses, hydroxyethyl celluloses, hydroxy propyl methyl celluloses, pectins, low-methoxy pectins, cross-linked dextrans, starch-acrylonitrile graft copolymers, starch sodium polyacrylate, hydroxyethyl methacrylates, hydroxyl ethyl acrylates, polyvinylene,

polyethylvinylethers, polymethyl methacrylates, polystyrenes, polyurethanes, polyalkanoates, polylactic acids, polylactates, poly(3-hydroxybutyrate), sulfonated hydrogels, AMPS (2-acrylamido-2-methyl-1-propanesulfonic acid), SEM (sulfoethylmethacrylate), SPM (sulfopropyl methacrylate), SPA (sulfopropyl acrylate), N,N-dimethyl-N-methacryloxyethyl-N-(3-sulfopropyl)ammonium betaine, methacryllic acid amidopropyl-dimethyl ammonium sulfobetaine, SPI (itaconic acid-bis(1-propyl sulfonizacid-3) ester di-potassium salt), itaconic acids, AMBC (3-acrylamido-3-methylbutanoic acid), beta-carboxyethyl acrylate (acrylic acid dimers), and maleic anhydride-methylvinyl ether polymers, derivatives thereof, salts thereof, acids thereof, and combinations thereof. In certain embodiments, pharmaceutical compositions of present disclosure can be administered orally, for example, in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis such as sucrose and acacia or tragacanth), powders, granules, a solution or a suspension in an aqueous or non-aqueous liquid, an oil-in-water or water-in-oil liquid emulsion, or an elixir or syrup, or pastille (using an inert base, such as gelatin and glycerin, or sucrose and acacia), and/or a mouth wash, each containing a predetermined amount of a compound of the present disclosure and optionally one or more other active ingredients. A compound of the present disclosure and optionally one or more other active ingredients may also be administered as a bolus, electuary, or paste.

In solid dosage forms for oral administration (*e.g.,* capsules, tablets, pills, dragees, powders, and granules), one or more compounds of the present disclosure may be mixed with one or more pharmaceutically acceptable carriers including, for example, sodium citrate, dicalcium phosphate, a filler or extender (*e.g.*, a starch, lactose, sucrose, glucose, mannitol, and silicic acid), a binder (*e.g.* carboxymethylcellulose, an alginate, gelatin, polyvinyl pyrrolidone, sucrose, and acacia), a humectant (*e.g.*, glycerol), a disintegrating agent (*e.g.*, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, a silicate, and sodium carbonate), a solution retarding agent (*e.g.* paraffin), an absorption accelerator (*e.g.* a quaternary ammonium compound), a wetting agent (*e.g.*, cetyl alcohol and glycerol monostearate), an absorbent (*e.g.*, kaolin and bentonite clay), a lubricant (*e.g.*, a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), a coloring agent, and mixtures thereof. In the case of capsules, tablets, and pills, the pharmaceutical formulation (composition) may also comprise a buffering agent. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using one or more excipients including, e.g., lactose or a milk sugar as well as a high molecular-weight polyethylene glycol.

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

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

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

In certain embodiments, it may be desirable to include an isotonic agent including, for example, a sugar or sodium chloride into the compositions. In addition, prolonged absorption of an injectable pharmaceutical form may be brought about by the inclusion of an agent that delay absorption including, for example, aluminum monostearate and gelatin.

It is understood that the dosage regimen will be determined by the attending physician considering various factors which modify the action of the one or more of the agents of the present disclosure. In the case of a TGF-beta superfamily heteromultimer 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 and embodiments of the present invention, and are not intended to limit the invention.

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

Applicants constructed a soluble ActRIIB-Fc:ALK4-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK4, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

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

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

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

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

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

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

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

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

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

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

```
   1  MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
  51  GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
 101  YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
 151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
 201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
 251  QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
 301  DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
```

351 SLSPG (SEQ ID NO: 104)

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

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

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC
TGTGTGGAGC

51 AGTCTTCGTT TCGCCCGGCG CCTCCGGGCC CCGGGGGGTC
CACGCTCTGC

101 TGTGTGCGTG CACCAGCTGC CTCCAGGCCA ACTACACGTG
TGAGACAGAT

151 GGGGCCTGCA TGGTTTCCAT TTTCAATCTG GATGGGATGG
AGCACCATGT

201 GCGCACCTGC ATCCCCAAAG TGGAGCTGGT CCCTGCCGGG
AAGCCCTTCT

251 ACTGCCTGAG CTCGGAGGAC CTGCGCAACA CCCACTGCTG
CTACACTGAC

301 TACTGCAACA GGATCGACTT GAGGGTGCCC AGTGGTCACC
TCAAGGAGCC

351 TGAGCACCCG TCCATGTGGG GCCCGGTGGA GACCGGTGGT
GGAACTCACA

401 CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC
GTCAGTCTTC

451 CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC
GGACCCCTGA

501 GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT
GAGGTCAAGT

551 TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA
GACAAAGCCG

601 CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG
TCCTCACCGT
651 CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC
AAGGTCTCCA
701 ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA
AGCCAAAGGG
751 CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC
GGGAGGAGAT
801 GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
TTCTATCCCA
851 GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA
GAACAACTAC
901 GACACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT
TCCTCTATAG
951 CGACCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
GTCTTCTCAT
1001 GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA
GAAGAGCCTC
1051 TCCCTGTCTC CGGGT (SEQ ID NO:105)

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

1 SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME
HHVRTCIPKV
51 ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL
KEPEHPSMWG
101 PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
TPEVTCVVVD
151 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
LTVLHQDWLN
201 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
EEMTKNQVSL
251 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
LYSDLTVDKS

301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G (SEQ ID NO: 106)

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

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

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

1 MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS

51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE

101 ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC

151 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV

201 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP

251 APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPSDIAV

301 EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH

351 EALHNHYTQK SLSLSPGK (SEQ ID NO: 401)

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

indicated by double underline above. The amino acid sequence of SEQ ID NO: 401 may optionally be provided with lysine removed from the C-terminus.

The mature ActRIIB-Fc fusion polypeptide is as follows:

1 GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT

51 IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA

101 GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS

151 RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS

201 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC

251 REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF

301 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK

(SEQ ID NO:402)

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

1 MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD

51 GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD

101 YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF

151 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP

201 REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG

251 QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY

301 KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL

351 SLSPGK (SEQ ID NO: 403)

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

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

1 SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV

51 ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG

101 PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD

151 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN

201 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL

251 SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS

301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 404)

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

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

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

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK4-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK4-Fc homodimeric complexes. The ActRIIB-Fc:ALK4-Fc heterodimer, ActRIIB-Fc homodimer, and ALK4-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

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

Ligand binding profile of ActRIIB-Fc:ALK4-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK4-Fc homodimer									
Ligand	ActRIIB-Fc homodimer			ALK4-Fc homodimer			ActRIIB-Fc:ALK4-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.2×10^7	2.3×10^{-4}	19	5.8×10^5	1.2×10^{-2}	20000	1.3×10^7	1.5×10^{-4}	12
Activin B	5.1×10^6	1.0×10^{-4}	20	No binding			7.1×10^6	4.0×10^{-5}	6

BMP6	3.2×10^{7}	6.8×10^{-3}	190	---			2.0×10^{6}	5.5×10^{-3}	2700
BMP9	1.4×10^{7}	1.1×10^{-3}	77	---			Transient*		3400
BMP10	2.3×10^{7}	2.6×10^{-4}	11	---			5.6×10^{7}	4.1×10^{-3}	74
GDF3	1.4×10^{6}	2.2×10^{-3}	1500	---			3.4×10^{6}	1.7×10^{-2}	4900
GDF8	8.3×10^{5}	2.3×10^{-4}	280	1.3×10^{5}	1.9×10^{-3}	15000†	3.9×10^{5}	2.1×10^{-4}	550
GDF11	5.0×10^{7}	1.1×10^{-4}	2	5.0×10^{6}	4.8×10^{-3}	270†	3.8×10^{7}	1.1×10^{-4}	3

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

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

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

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

Homodimeric and heterodimeric complexes were tested in mice to investigate differences in their activity profiles in vivo. Wild-type C57BL/6 mice were dosed subcutaneously with an ActRIIB-Fc homodimer (10 mg/kg), an ActRIIB-Fc:ALK4-Fc

heterodimer (3 or 10 mg/kg), or vehicle (phosphate-buffered saline, PBS) twice per week for 4 weeks beginning at approximately 10 weeks of age (n = 9 mice per group). ALK4-Fc homodimer was not tested in vivo due to its inability to bind ligands with high affinity under cell-free conditions as determined by surface plasmon resonance. Study endpoints included: body weight; total lean mass and total adipose mass as determined by nuclear magnetic resonance (NMR) at baseline and study completion (4 weeks); total bone mineral density as determined by dual energy x-ray absorptiometry (DEXA) at baseline and 4 weeks; and weights of the gastrocnemius, rectus femoris, and pectoralis muscles determined at 4 weeks.

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

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

adipose mass (typically about 10% of body weight). Individual skeletal muscles examined, including the gastrocnemius, femoris, and pectoralis all increased significantly in weight compared to vehicle controls over the course of treatment with ActRIIB-Fc homodimer.

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

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

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

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

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

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

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

1 MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS
DQKKSENCVT
51 LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED
DQGETTLASG
101 CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP
PVVIGPFFDG
151 SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
TPEVTCVVVD
201 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
LTVLHQDWLN
251 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
EEMTKNQVSL
301 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
LYSDLTVDKS
351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G (SEQ ID NO: 115)

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

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

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC
TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCG CCCAGAATCT GGATAGTATG
CTTCATGGCA
101 CTGGGATGAA ATCAGACTCC GACCAGAAAA AGTCAGAAAA
TGGAGTAACC
151 TTAGCACCAG AGGATACCTT GCCTTTTTTA AAGTGCTATT
GCTCAGGGCA
201 CTGTCCAGAT GATGCTATTA ATAACACATG CATAACTAAT
GGACATTGCT

251 TTGCCATCAT AGAAGAAGAT GACCAGGGAG AAACCACATT AGCTTCAGGG

301 TGTATGAAAT ATGAAGGATC TGATTTTCAG TGCAAAGATT CTCCAAAAGC

351 CCAGCTACGC CGGACAATAG AATGTTGTCG GACCAATTTA TGTAACCAGT

401 ATTTGCAACC CACACTGCCC CCTGTTGTCA TAGGTCCGTT TTTTGATGGC

451 AGCATTCGAA CCGGTGGTGG AACTCACACA TGCCCACCGT GCCCAGCACC

501 TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG

551 ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC

601 GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT

651 GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA

701 CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT

751 GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT

801 CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT

851 ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG

901 ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA

951 GAGCAATGGG CAGCCGGAGA ACAACTACGA CACCACGCCT CCCGTGCTGG

1001 ACTCCGACGG CTCCTTCTTC CTCTATAGCG ACCTCACCGT GGACAAGAGC

1051 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT

1101 GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGT
(SEQ ID NO: 116)

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

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

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

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

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

1 MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
51 LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
101 CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG

151 SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
301 SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS
351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 407)

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

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

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

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

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

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

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

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

Ligand	ActRIIB-Fc homodimer			ALK3-Fc homodimer			ActRIIB-Fc:ALK3-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.3×10^7	1.4×10^{-4}	11	No binding			3.4×10^7	5.0×10^{-3}	150
Activin B	5.1×10^6	1.0×10^{-4}	20	No binding			2.8×10^6	5.7×10^{-4}	200
BMP2	Transient*		> 66000	6.8×10^5	8.9×10^{-5}	130	8.0×10^6	1.1×10^{-5}	1
BMP4	---			3.0×10^5	5.3×10^{-5}	180	2.6×10^6	6.5×10^{-6}	3
BMP5	2.6×10^7	7.5×10^{-2}	2900	2.9×10^4	2.0×10^{-3}	70000	9.0×10^5	5.8×10^{-4}	640
BMP6	3.5×10^7	6.8×10^{-3}	190	1.4×10^5	4.9×10^{3}	35000	2.0×10^7	2.9×10^{-4}	15
BMP7	8.8×10^6	1.4×10^{-2}	1600	1.2×10^6	1.8×10^{-2}	15000	8.2×10^5	1.5×10^{-3}	1900
BMP9	3.9×10^7	1.3×10^{-3}	34	No binding			Transient*		>33000
BMP10	5.9×10^7	2.0×10^{-4}	4	No binding			3.0×10^7	9.4×10^{-4}	31
GDF3	1.6×10^6	2.3×10^{-3}	1400	No binding			1.4×10^7	8.2×10^{-2}	5900
GDF5	Transient*		> 9600	4.8×10^5	1.1×10^{-2}	22000	1.2×10^7	8.3×10^{-4}	70
GDF6	---			3.4×10^4	1.3×10^{-3}	40000	2.8×10^5	4.5×10^{-4}	1600
GDF7	Transient*		> 12000	2.2×10^5	2.7×10^{-2}	12000	7.5×10^6	4.0×10^{-4}	52
GDF8	8.3×10^5	2.3×10^{-4}	280	No binding			3.0×10^6	9.2×10^{-4}	310
GDF11	5.0×10^7	1.1×10^{-4}	2	No binding			1.6×10^7	1.1×10^{-3}	66

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

These comparative binding data demonstrate that the ActRIIB-Fc:ALK3-Fc heterodimer has an altered binding profile/selectivity relative to either the ActRIIB-Fc homodimer or ALK3-Fc homodimer. The ActRIIB-Fc:ALK3-Fc heterodimer binds BMP2

and BMP4 with exceptionally high affinity and displays greatly enhanced binding to BMP5, BMP6, BMP7, GDF5, GDF6, and GDF7 compared with either homodimer. Compared to ActRIIB homodimer, the ActRIIB-Fc:ALK3-Fc heterodimer displays reduced binding to activin A, activin B, BMP10, GDF8, and GDF11 and also discriminates among these ligands to a greater degree, particularly between activin A and activin B. In addition, the ability of ActRIIB-Fc homodimer to bind BMP9 and GDF3 with high affinity is absent for ActRIIB-Fc:ALK3-Fc heterodimer. See **Figure 7**.

These results therefore demonstrate that the ActRIIB-Fc:ALK3-Fc heterodimer is a selective inhibitor of activin B, the GDF5/GDF6/GDF7 ligand subfamily, and several key BMP ligands excluding most notably BMP9. Accordingly, an ActRIIB-Fc:ALK3-Fc heterodimer will be more useful than either an ActRIIB-Fc homodimer or an ALK3-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of BMP2, BMP4, BMP5, and BMP6 or activin B but minimize antagonism of one or more ligands with anabolic muscle effects (e.g., activin A and GDF8) or ligands with angiogenic effects (e.g., BMP9 and BMP10).

Example 6. Activity profile of ActRIIB-Fc:ALK3-Fc heterodimer in mice compared to ActRIIB-Fc homodimer and ALK3-Fc homodimer

Homodimeric and heterodimeric complexes were tested in mice to investigate differences in their activity profiles in vivo. Wild-type C57BL/6 mice were dosed intraperitoneally with ActRIIB-Fc homodimer (10 mg/kg), ALK3-Fc homodimer (10 mg/kg), ActRIIB-Fc:ALK4-Fc heterodimer (3 or 10 mg/kg), or vehicle (phosphate-buffered saline, PBS) twice per week for 6.5 weeks (46 days) beginning at 10 weeks of age (n = 5 mice per group). Study endpoints included body weight, total adipose mass as determined by nuclear magnetic resonance (NMR) at baseline and study completion (6.5 weeks), and total bone mineral density as determined by dual energy x-ray absorptiometry (DEXA) at baseline and 6.5 weeks.

Activity of ActRIIB-Fc and ALK3-Fc Complexes in Wild-Type Mice Compared to Vehicle			
Endpoint 6.5 wk	ActRIIB-Fc homodimer	ALK3-Fc homodimer	ActRIIB-Fc:ALK3-Fc heterodimer

	10 mg/kg	10 mg/kg	10 mg/kg	3 mg/kg
Body weight	↑ 23% *	↓ 3%	↓ 0.5%	↓ 1%
Total adipose mass	↓ 41% *	↓ 12%	↓ 14% *	↓ 18% *
Total bone mineral density	↑ 8% *	↑ 6% *	↑ 9% *	↑ 10% *
*$P < 0.05$ vs. vehicle				

Study results are summarized in the table above. As expected, the ActRIIB-Fc homodimer significantly increased body weight and total bone mineral density, and significantly reduced total adipose mass, all compared to vehicle. Also as expected, the ALK3-Fc homodimer significantly increased total bone mineral density compared to vehicle but unlike the ActRIIB-Fc homodimer did not significantly alter either body weight or total adipose mass. The ActRIIB-Fc:ALK3-Fc heterodimer notably displayed an activity profile different from either the ActRIIB-Fc homodimer or the ALK3-Fc homodimer. Treatment of mice with the ActRIIB-Fc:ALK4-Fc heterodimer at either dose level significantly increased bone mineral density at least as well either homodimer. However, unlike ALK3-Fc homodimer, the ActRIIB-Fc:ALK3-Fc heterodimer significantly reduced adipose mass, and unlike ActRIIB-Fc homodimer, the ActRIIB-Fc:ALK3-Fc heterodimer signficantly reduced adipose mass without altering body weight. Thus, an ActRIIB-Fc:ALK3-Fc heterodimer exerts beneficial effects on bone together with potentially beneficial effects on adipose tissue. This novel selectivity will be useful, for example, in treating patients in need of stimulatory effects on bone and inhibitory effects on fat but not in need of altered body weight.

Example 7. Generation of an ActRIIB-Fc:ALK7-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK7-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK7, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK7-Fc, respectively.

Formation of heteromeric ALK7-Fc:ActRIIB-Fc may be guided by approaches similar to those described in Example 1.

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

The complementary ALK7-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 112):

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251  EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
301  LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
       (SEQ ID NO: 112)
```

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK7-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 112 may optionally be provided with a lysine added at the C-terminus.

This ALK7-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 113):

```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51  AGTCTTCGTT TCGCCCGGCG CCGGACTGAA GTGTGTATGT CTTTTGTGTG
101  ATTCTTCAAA CTTTACCTGC CAAACAGAAG GAGCATGTTG GGCATCAGTC
151  ATGCTAACCA ATGGAAAAGA GCAGGTGATC AAATCCTGTG TCTCCCTTCC
201  AGAACTGAAT GCTCAAGTCT TCTGTCATAG TTCCAACAAT GTTACCAAAA
251  CCGAATGCTG CTTCACAGAT TTTTGCAACA ACATAACACT GCACCTTCCA
301  ACAGCATCAC CAAATGCCCC AAAACTTGGA CCCATGGAGA CCGGTGGTGG
351  AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG GGGGGACCGT
401  CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG
451  ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA
501  GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA
551  CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC
601  CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA
651  GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG
701  CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG
```

```
 751  GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT
 801  CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA
 851  ACAACTACGA CACCACGCCT CCCGTGCTGG ACTCCGACGG CTCCTTCTTC
 901  CTCTATAGCG ACCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT
 951  CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA
1001  AGAGCCTCTC CCTGTCTCCG GGT        (SEQ ID NO: 113)
```

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 114) is expected to be as follows and may optionally be provided with a lysine added at the C-terminus.

```
  1  GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51  CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
201  APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
251  EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ GNVFSCSVMH
301  EALHNHYTQK SLSLSPG          (SEQ ID NO: 114)
```

The ActRIIB-Fc and ALK7-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 114, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK7-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK7-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 405-406, respectively, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK7-Fc fusion polypeptide (SEQ ID NO: 405) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR
251  EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301  LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
```

(SEQ ID NO: 405)

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK7 fusion polypeptide as indicated by double underline above. Furthermore, the C-terminal lysine residue of the Fc domain can be deleted. The amino acid sequence of SEQ ID NO: 405 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 406) is expected to be as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51  CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
201  APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV KGFYPSDIAV
251  EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH
301  EALHNHYTQK SLSLSPGK        (SEQ ID NO: 406)
```

The ActRIIB-Fc and ALK7-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 406, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK7-Fc.

Purification of various ActRIIB-Fc:ALK7-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 8. Ligand binding profile of ActRIIB-Fc:ALK7-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK7-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK7-Fc homodimeric complexes. The ActRIIB-Fc:ALK7-Fc heterodimer, ActRIIB-Fc

homodimer, and ALK7-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

5

Ligand binding profile of ActRIIB-Fc:ALK7-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer									
Ligand	ActRIIB-Fc homodimer			ALK7-Fc homodimer			ActRIIB-Fc:ALK7-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.3×10^7	1.4×10^{-4}	11	No binding			4.4×10^7	1.9×10^{-3}	43
Activin B	1.5×10^7	1.6×10^{-4}	8	No binding			1.2×10^7	2.0×10^{-4}	17
BMP5	2.6×10^7	7.5×10^{-2}	2900	No binding			1.5×10^5	8.5×10^{-3}	57000
BMP6	2.4×10^7	3.9×10^{-3}	160	No binding			1.2×10^6	6.3×10^{-3}	5300
BMP9	1.2×10^8	1.2×10^{-3}	10	No binding			Transient*		>1400
BMP10	5.9×10^6	1.5×10^{-4}	25	No binding			1.5×10^7	2.8×10^{-3}	190
GDF3	1.4×10^6	2.2×10^{-3}	1500	No binding			2.3×10^6	1.0×10^{-2}	4500
GDF8	3.5×10^6	2.4×10^{-4}	69	No binding			3.7×10^6	1.0×10^{-3}	270
GDF11	9.6×10^7	1.5×10^{-4}	2	No binding			9.5×10^7	7.5×10^{-4}	8
* Indeterminate due to transient nature of interaction									

These comparative binding data demonstrate that the ActRIIB-Fc:ALK7-Fc heterodimer has a different binding profile compared to either the ActRIIB-Fc homodimer or ALK7-Fc homodimer. Interestingly, four of the five ligands with strong binding to ActRIIB-Fc homodimer (activin A, BMP10, GDF8, and GDF11) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer, the exception being activin B which retains tight binding to the heterodimer. In addition, three ligands with intermediate binding to ActRIIB-Fc homodimer (GDF3, BMP6, and particularly BMP9) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer. In contrast, BMP5 binds the ActRIIB-Fc:ALK7 heterodimer with

10

intermediate strength despite only weak binding to ActRIIB-Fc homodimer. No ligands tested bind to ALK7-Fc homodimer. See **Figure 8**.

These results therefore demonstrate that the ActRIIB-Fc:ALK7-Fc heterodimer is a more selective antagonist of activin B in comparison to a ActRIIB-Fc homodimer. Accordingly, an ActRIIB-Fc:ALK7-Fc heterodimer will be more useful than an ActRIIB-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of activin B but minimize antagonism of one or more of activin A, GDF3, GDF8, GDF11, BMP9, or BMP10.

Example 9. Generation of an ActRIIB-Fc:ALK2-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK2-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK2, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK2-Fc, respectively.

Formation of heteromeric ActRIIB-Fc:ALK2-Fc may be guided by approaches similar to those described in Example 1.

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

The complementary ALK2-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 136):

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
301  DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPG      (SEQ ID NO: 136)
```

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK2-Fc heterodimer rather than either of the possible homodimeric complexes,

two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 136 may optionally be provided with a lysine added at the C-terminus.

This ALK2-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 137):

```
   1   ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC
TGTGTGGAGC
  51   AGTCTTCGTT TCGCCCGGCG CCATGGAAGA TGAGAAGCCC
AAGGTCAACC
 101   CCAAACTCTA CATGTGTGTG TGTGAAGGTC TCTCCTGCGG
TAATGAGGAC
 151   CACTGTGAAG GCCAGCAGTG CTTTTCCTCA CTGAGCATCA
ACGATGGCTT
 201   CCACGTCTAC CAGAAAGGCT GCTTCCAGGT TTATGAGCAG
GGAAAGATGA
 251   CCTGTAAGAC CCCGCCGTCC CCTGGCCAAG CTGTGGAGTG
CTGCCAAGGG
 301   GACTGGTGTA ACAGGAACAT CACGGCCCAG CTGCCCACTA
AAGGAAAATC
 351   CTTCCCTGGA ACACAGAATT TCCACTTGGA GACCGGTGGT
GGAACTCACA
 401   CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC
GTCAGTCTTC
 451   CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC
GGACCCCTGA
 501   GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT
GAGGTCAAGT
 551   TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA
GACAAAGCCG
 601   CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG
TCCTCACCGT
```

651 CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC
AAGGTCTCCA
701 ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA
AGCCAAAGGG
751 CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC
GGGAGGAGAT
801 GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
TTCTATCCCA
851 GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA
GAACAACTAC
901 GACACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT
TCCTCTATAG
951 CGACCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
GTCTTCTCAT
1001 GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA
GAAGAGCCTC
1051 TCCCTGTCTC CGGGT (SEQ ID NO: 137)

The mature ALK2-Fc fusion protein sequence (SEQ ID NO: 138) is as follows and may optionally be provided with a lysine added at the C-terminus.

1 MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
51 FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
101 HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
251 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF LYSDLTVDKS
301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G (SEQ ID NO: 138)

The ActRIIB-Fc and ALK2-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 138, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK2-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK2-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 421-422, respectively, the Fc domains are altered to

introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK2-Fc fusion polypeptide (SEQ ID NO: 421) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY
301  KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPGK        (SEQ ID NO: 421)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK2 fusion polypeptide as indicated by double underline above. Furthermore, the C-terminal lysine residue of the Fc domain can be deleted. The amino acid sequence of SEQ ID NO: 421 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK2-Fc fusion protein sequence (SEQ ID NO: 422) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
 51  FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
101  HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
251  SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK   (SEQ ID NO: 422)
```

The ActRIIB-Fc and ALK2-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 422, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK2-Fc.

Purification of various ActRIIB-Fc:ALK2-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the

following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

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Example 10. Ligand binding profile of ActRIIB-Fc:ALK2-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK2-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK2-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK2-Fc homodimeric complexes. The ActRIIB-Fc:ALK2-Fc heterodimer, ActRIIB-Fc homodimer, and ALK2-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

10

Ligand binding profile of ActRIIB-Fc:ALK2-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK2-Fc homodimer									
Ligand	ActRIIB-Fc Homodimer			ALK2-Fc Homodimer			ActRIIB-Fc:ALK2-Fc Heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.2×10^7	1.7×10^{-4}	15	No binding			3.4×10^7	2.6×10^{-3}	76
Activin B	3.8×10^6	1.1×10^{-4}	28	No binding			3.2×10^6	1.5×10^{-4}	47
BMP5	3.8×10^6	3.7×10^{-2}	9700	No binding			1.2×10^6	1.4×10^{-3}	1200
BMP7	8.8×10^6	1.4×10^{-2}	1600	No binding			1.5×10^7	2.6×10^{-3}	170
BMP9	3.9×10^7	1.3×10^{-3}	34	No binding			3.2×10^6	8.9×10^{-4}	280
BMP10	5.4×10^7	2.8×10^{-4}	5	No binding			5.5×10^7	2.9×10^{-3}	53
GDF3	1.2×10^6	2.0×10^{-3}	1700	No binding			1.8×10^6	1.2×10^{-2}	6500
GDF5	1.2×10^6	1.4×10^{-3}	1100	No binding			8.8×10^5	4.4×10^{-3}	5000
GDF6	1.5×10^5	5.7×10^{-3}	39000	No binding			Transient*		> 240000
GDF8	2.5×10^6	3.2×10^{-4}	130	No binding			2.1×10^6	7.3×10^{-4}	360
GDF11	2.0×10^6	2.2×10^{-4}	110	No binding			1.6×10^6	9.3×10^{-4}	600
* Indeterminate due to transient nature of interaction									

These comparative binding data demonstrate that the ActRIIB-Fc:ALK2-Fc heterodimer exhibits a ligand binding profile different from either the ActRIIB-Fc homodimer or the ALK2-Fc homodimer. ActRIIB-Fc:ALK2-Fc heterodimer exhibits preferential and strong binding to activin B, thus resembling ActRIIB-Fc:ALK7-Fc heterodimer (see Example 8). However, ActRIIB-Fc:ALK2-Fc heterodimer differs from ActRIIB-Fc:ALK7-Fc in part by retaining the tight binding to BMP9 characteristic of ActRIIB-Fc homodimer, whereas ActRIIB-Fc:ALK7-Fc binds BMP9 very weakly, if at all. No ligands tested bind to ALK2-Fc homodimer. **See Figure 9**.

These results demonstrate that the ActRIIB-Fc:ALK2-Fc heterodimer is a more selective antagonist of activin B compared to ActRIIB-Fc homodimer. Accordingly, an ActRIIB-Fc:ALK2-Fc heterodimer will be useful in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism primarily of activin B and to supplement that with antagonism secondarily of BMP9, GDF8, and GDF11.

Example 11. Generation of an ActRIIB-Fc:ALK5-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK5-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK5, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK5-Fc, respectively.

Formation of heteromeric ActRIIB-Fc: ALK5-Fc may be guided by approaches similar to those described in Example 1.

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

The complementary ALK5-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 139):

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
 51  GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
101  NQDHCNKIEL PTTVKSSPGL GPVETGGGTH TCPPCPAPEL LGGPSVFLFP
151  PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
201  QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
```

```
251  EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYDTT
301  PPVLDSDGSF FLYSDLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
351  PG   (SEQ ID NO: 139)
```

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 139 may optionally be provided with a lysine added at the C-terminus.

This ALK5-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 140):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGCGCTGCT CCCGGGGGCG ACGGCGTTAC
 101  AGTGTTTCTG CCACCTCTGT ACAAAAGACA ATTTTACTTG TGTGACAGAT
 151  GGGCTCTGCT TTGTCTCTGT CACAGAGACC ACAGACAAAG TTATACACAA
 201  CAGCATGTGT ATAGCTGAAA TTGACTTAAT TCCTCGAGAT AGGCCGTTTG
 251  TATGTGCACC CTCTTCAAAA ACTGGGTCTG TGACTACAAC ATATTGCTGC
 301  AATCAGGACC ATTGCAATAA AATAGAACTT CCAACTACTG TAAAGTCATC
 351  ACCTGGCCTT GGTCCTGTGG AAACCGGTGG TGGAACTCAC ACATGCCCAC
 401  CGTGCCCAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC
 451  CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG
 501  CGTGGTGGTG GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT
 551  ACGTGGACGG CGTGGAGGTG CATAATGCCA AGACAAAGCC GCGGGAGGAG
 601  CAGTACAACA GCACGTACCG TGTGGTCAGC GTCCTCACCG TCCTGCACCA
 651  GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC AACAAAGCCC
 701  TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
 751  GAACCACAGG TGTACACCCT GCCCCCATCC CGGGAGGAGA TGACCAAGAA
 801  CCAGGTCAGC CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG
 851  CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG AGAACAACTA CGACACCACG
 901  CCTCCCGTGC TGGACTCCGA CGGCTCCTTC TTCCTCTATA GCGACCTCAC
 951  CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA
1001  TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
1051  CCGGGT   (SEQ ID NO: 140)
```

The mature ALK5-Fc fusion protein sequence (SEQ ID NO: 141) is as follows and may optionally be provided with a lysine added at the C-terminus.

```
  1  ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
 51  DLIPRDRPFV CAPSSKTGSV TTTYCCNQDH CNKIELPTTV KSSPGLGPVE
101  TGGGTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
151  EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
201  YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL
251  VKGFYPSDIA VEWESNGQPE NNYDTTPPVL DSDGSFFLYS DLTVDKSRWQ
301  QGNVFSCSVM HEALHNHYTQ KSLSLSPG   (SEQ ID NO: 141)
```

The ActRIIB-Fc and ALK5-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 141, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK5-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK5-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 423-424, respectively, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK5-Fc fusion polypeptide (SEQ ID NO: 423) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
 51  GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
101  NQDHCNKIEL PTTVKSSPGL GPVETGGGTH TCPPCPAPEL LGGPSVFLFP
151  PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
201  QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
251  EPQVCTLPPS REEMTKNQVS LSCAVKGFYP SDIAVEWESN GQPENNYKTT
301  PPVLDSDGSF FLVSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
351  PGK      (SEQ ID NO: 423)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK5 fusion polypeptide as indicated by double underline above. Furthermore, the C-terminal lysine

residue of the Fc domain can be deleted. The amino acid sequence of SEQ ID NO: 423 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK5-Fc fusion protein sequence (SEQ ID NO: 424) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
 51  DLIPRDRPFV CAPSSKTGSV TTTYCCNQDH CNKIELPTTV KSSPGLGPVE
101  TGGGTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
151  EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
201  YKCKVSNKAL PAPIEKTISK AKGQPREPQV CTLPPSREEM TKNQVSLSCA
251  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLVS KLTVDKSRWQ
301  QGNVFSCSVM HEALHNHYTQ KSLSLSPGK      (SEQ ID NO: 424)
```

The ActRIIB-Fc and ALK5-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 424, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK5-Fc.

Purification of various ActRIIB-Fc:ALK5-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 12. Ligand binding profile of ActRIIB-Fc:ALK5-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK5-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK5-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK5-Fc homodimeric complexes. The ActRIIB-Fc:ALK5-Fc heterodimer, ActRIIB-Fc homodimer, and ALK5-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of ActRIIB-Fc:ALK5-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK5-Fc homodimer									
Ligand	ActRIIB-Fc Homodimer			ALK5-Fc Homodimer			ActRIIB-Fc:ALK5-Fc Heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.2×10^7	2.3×10^{-4}	19	No binding			3.6×10^7	1.6×10^{-3}	46
Activin B	5.1×10^6	1.0×10^{-4}	20	No binding			3.9×10^6	3.1×10^{-4}	79
BMP6	6.4×10^6	7.0×10^{-3}	1100	No binding			9.3×10^6	1.5×10^{-2}	1700
BMP9	3.9×10^7	1.3×10^{-3}	34	No binding			Transient*		> 6600
BMP10	2.1×10^7	3.8×10^{-4}	18	No binding			2.3×10^7	2.2×10^{-3}	150
GDF3	4.7×10^5	1.8×10^{-3}	3900	No binding			1.1×10^5	9.7×10^{-3}	8500
GDF8	1.2×10^6	1.9×10^{-4}	160	No binding			1.1×10^6	5.2×10^{-4}	490
GDF11	1.9×10^6	1.4×10^{-4}	74	No binding			2.3×10^6	4.6×10^{-4}	600
* Indeterminate due to transient nature of interaction									

Example 13. Generation of an ActRIIB-Fc:ALK6-Fc heterodimer

A soluble ActRIIB-Fc:ALK6-Fc heteromeric complex can be generated comprising the extracellular domains of human ActRIIB and human ALK6, which can each be fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK6-Fc, respectively.

Formation of heteromeric ActRIIB-Fc: ALK6-Fc may be guided by approaches similar to those described in Example 1.

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

The complementary ALK6-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 142):

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51  EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLEGSDF QCRDTPIPHQ
101  RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
```

```
251  IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW
301  ESNGQPENNY DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA
351  LHNHYTQKSL SLSPG      (SEQ ID NO: 142)
```

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK6-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 142 may optionally be provided with a lysine added at the C-terminus.

This ALK6-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 143):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCAAGAAAGA GGATGGTGAG AGTACAGCCC
 101  CCACCCCCCG TCCAAAGGTC TTGCGTTGTA AATGCCACCA CCATTGTCCA
 151  GAAGACTCAG TCAACAATAT TTGCAGCACA GACGGATATT GTTTCACGAT
 201  GATAGAAGAG GATGACTCTG GGTTGCCTGT GGTCACTTCT GGTTGCCTAG
 251  GACTAGAAGG CTCAGATTTT CAGTGTCGGG ACACTCCCAT TCCTCATCAA
 301  AGAAGATCAA TTGAATGCTG CACAGAAAGG AACGAATGTA ATAAAGACCT
 351  ACACCCTACA CTGCCTCCAT TGAAAAACAG AGATTTTGTT GATGGACCTA
 401  TACACCACAG GACCGGTGGT GGAACTCACA CATGCCCACC GTGCCCAGCA
 451  CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA
 501  GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG
 551  ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC
 601  GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG
 651  CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA
 701  ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC
 751  ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT
 801  GTACACCCTG CCCCCATCCC GGGAGGAGAT GACCAAGAAC CAGGTCAGCC
 851  TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
 901  GAGAGCAATG GGCAGCCGGA GAACAACTAC GACACCACGC CTCCCGTGCT
 951  GGACTCCGAC GGCTCCTTCT TCCTCTATAG CGACCTCACC GTGGACAAGA
1001  GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT
1051  CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGT
```

(SEQ ID NO: 143)

The mature ALK6-Fc fusion protein sequence (SEQ ID NO: 144) is as follows and may optionally be provided with a lysine added at the C-terminus.

```
  1  KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
 51  LPVVTSGCLG LEGSDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101  KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251  EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
301  LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
     (SEQ ID NO: 144)
```

The ActRIIB-Fc and ALK6-Fc fusion proteins of SEQ ID NO: 102 and SEQ ID NO: 144, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK6-Fc.

In another approach to promoting the formation of heteromultimer complexes using asymmetric Fc fusion proteins, illustrated in the ActRIIB-Fc and ALK6-Fc polypeptide sequences of SEQ ID NOs: 401-402 and 425-426, respectively, the Fc domains can be altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. The ActRIIB-Fc fusion polypeptide sequences are discussed in Example 1.

The complementary form of ALK6-Fc fusion polypeptide (SEQ ID NO: 425) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51  EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLEGSDF QCRDTPIPHQ
101  RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251  IEKTISKAKG QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW
301  ESNGQPENNY KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA
351  LHNHYTQKSL SLSPGK        (SEQ ID NO: 425)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401 and 402 above, four amino acid substitutions can be introduced into the Fc domain of the ALK6 fusion

polypeptide as indicated by double underline above. Furthermore, the C-terminal lysine residue of the Fc domain can be deleted. The amino acid sequence of SEQ ID NO: 425 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK6-Fc fusion protein sequence (SEQ ID NO: 426) can be as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
 51  LPVVTSGCLG LEGSDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101  KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR
251  EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301  LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
     (SEQ ID NO: 426)
```

The ActRIIB-Fc and ALK6-Fc proteins of SEQ ID NO: 402 and SEQ ID NO: 426, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK6-Fc.

Purification of various ActRIIB-Fc:ALK6-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 14. Generation of an ActRIIA-Fc:ALK4-Fc heterodimer

Applicants constructed a soluble ActRIIA-Fc:ALK4-Fc heteromeric complex comprising the extracellular domains of human ActRIIA and human ALK4, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIA-Fc fusion polypeptide and ALK4-Fc fusion polypeptide, respectively.

Formation of heteromeric ActRIIA-Fc:ALK4-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to

introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face.

The ActRIIA-Fc polypeptide sequence (SEQ ID NO: 118) is shown below:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51 GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101 DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151 CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201 VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251 PAPIEKTISK AKGQPREPQV YTLPPSRKEM TKNQVSLTCL VKGFYPSDIA
301 VEWESNGQPE NNYKTTPPVL KSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
351 HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 118)
```

The leader sequence and linker sequence are underlined. To promote formation of the ActRIIA-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the ActRIIA fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 118 may optionally be provided with the lysine removed from the C-terminus.

This ActRIIA-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 119):

```
   1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51 AGTCTTCGTT TCGCCCGGCG CCGCTATACT TGGTAGATCA GAAACTCAGG
 101 AGTGTCTTTT CTTTAATGCT AATTGGGAAA AAGACAGAAC CAATCAAACT
 151 GGTGTTGAAC CGTGTTATGG TGACAAAGAT AAACGGCGGC ATTGTTTTGC
 201 TACCTGGAAG AATATTTCTG GTTCCATTGA AATAGTGAAA CAAGGTTGTT
 251 GGCTGGATGA TATCAACTGC TATGACAGGA CTGATTGTGT AGAAAAAAAA
 301 GACAGCCCTG AAGTATATTT CTGTTGCTGT GAGGGCAATA TGTGTAATGA
 351 AAAGTTTTCT TATTTTCCGG AGATGGAAGT CACACAGCCC ACTTCAAATC
 401 CAGTTACACC TAAGCCACCC ACCGGTGGTG GAACTCACAC ATGCCCACCG
 451 TGCCCAGCAC CTGAACTCCT GGGGGGACCG TCAGTCTTCC TCTTCCCCCC
 501 AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACATGCG
 551 TGGTGGTGGA CGTGAGCCAC GAAGACCCTG AGGTCAAGTT CAACTGGTAC
 601 GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCGC GGGAGGAGCA
```

```
 651  GTACAACAGC ACGTACCGTG TGGTCAGCGT CCTCACCGTC CTGCACCAGG
 701  ACTGGCTGAA TGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGCCCTC
 751  CCAGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAAAGGGC AGCCCCGAGA
 801  ACCACAGGTG TACACCCTGC CCCCATCCCG GAAGGAGATG ACCAAGAACC
 851  AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTATCCCAG CGACATCGCC
 901  GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACTACA AGACCACGCC
 951  TCCCGTGCTG AAGTCCGACG GCTCCTTCTT CCTCTATAGC AAGCTCACCG
1001  TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
1051  CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
1101  GGGTAAA                    (SEQ ID NO: 119)
```

The mature ActRIIA-Fc fusion polypeptide (SEQ ID NO: 120) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51  IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101  EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151  SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201  SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
251  SRKEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLKSDGS
301  FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
     (SEQ ID NO: 120)
```

In this first approach, the polypeptide sequence of the complementary ALK4-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 1 as SEQ ID NOs: 104-106.

The ActRIIA-Fc and ALK4-Fc proteins of SEQ ID NO: 120 and SEQ ID NO: 106, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIA-Fc:ALK4-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond.

The ActRIIA-Fc polypeptide sequence (SEQ ID NO: 409) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51  GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
```

```
101  DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151  CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201  VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251  PAPIEKTISK AKGQPREPQV YTLPPCREEM TKNQVSLWCL VKGFYPSDIA
301  VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
351  HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 409)
```

The leader sequence and linker sequence are underlined. To promote formation of the ActRIIA-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 409 may optionally be provided with the lysine removed from the C-terminus.

The mature ActRIIA-Fc fusion polypeptide (SEQ ID NO: 410) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51  IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101  EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151  SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201  SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
251  CREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
301  FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
     (SEQ ID NO: 410)
```

In this second approach, the polypeptide sequence of the complementary ALK4-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 1 as SEQ ID NOs: 403-404.

The ActRIIA-Fc and ALK4-Fc proteins of SEQ ID NO: 410 and SEQ ID NO: 404, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIA-Fc:ALK4-Fc.

Purification of various ActRIIA-Fc:ALK4-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange

chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 15. Ligand binding profile of ActRIIA-Fc:ALK4-Fc heterodimer compared to ActRIIA-Fc homodimer and ALK4-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIA-Fc:ALK4-Fc heterodimeric complex described above with that of ActRIIA-Fc and ALK4-Fc homodimeric complexes. The ActRIIA-Fc:ALK4-Fc heterodimer, ActRIIA-Fc homodimer, and ALK4-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of ActRIIA-Fc:ALK4-Fc heterodimer compared to ActRIIA-Fc homodimer and ALK4-Fc homodimer

Ligand	ActRIIA-Fc homodimer			ALK4-Fc homodimer			ActRIIA-Fc:ALK4-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.4×10^7	6.2×10^{-4}	45	5.8×10^5	1.2×10^{-2}	20000	7.4×10^6	2.4×10^{-4}	32
Activin B	1.1×10^7	1.1×10^{-4}	10	No binding			9.5×10^6	4.8×10^{-4}	50
Activin AB	2.8×10^7	2.6×10^{-4}	9	1.8×10^6	3.6×10^{-3}	2000	1.8×10^7	2.3×10^{-4}	13
Activin AC	2.2×10^7	7.9×10^{-3}	360	No binding			3.2×10^6	5.4×10^{-4}	170
BMP6	2.7×10^8	2.2×10^{-2}	800	No binding			5.4×10^6	1.2×10^{-2}	2200
BMP7	8.9×10^6	3.3×10^{-2}	3700	No binding			2.0×10^7	7.2×10^{-2}	3500
BMP9	Transient*		>10000	---			No binding		
BMP10	2.9×10^7	2.5×10^{-3}	85	No binding			Transient*		>6000
GDF3	1.5×10^6	3.6×10^{-3}	2400	---			4.9×10^7	4.8×10^{-3}	9800
GDF8	1.4×10^6	1.4×10^{-3}	99	1.3×10^5	1.9×10^{-3}	15000†	1.8×10^7	2.8×10^{-3}	150
GDF11	7.3×10^7	9.2×10^{-4}	13	5.0×10^6	4.8×10^{-3}	970†	3.0×10^7	6.5×10^{-4}	22

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

These comparative binding data demonstrate that the ActRIIA-Fc:ALK4-Fc heterodimer has an altered binding profile/selectivity relative to either the ActRIIA-Fc or ALK4-Fc homodimers. For example, the ActRIIA-Fc:ALK4-Fc heterodimer exhibits enhanced binding to activin A, and particularly enhanced binding to activin AC, compared to ActRIIA-Fc homodimer, while retaining strong binding to activin AB and GDF11. In addition, the ligand with highest affinity for ActRIIA-Fc homodimer, activin B, displays reduced affinity (albeit still within the high-affinity range) for the ActRIIA-Fc:ALK4-Fc heterodimer. The ActRIIA-Fc:ALK4-Fc heterodimer also exhibits markedly reduced binding to BMP10 compared to ActRIIA-Fc homodimer. See **Figure 10**.

These results demonstrate that the ActRIIA-Fc:ALK4-Fc heterodimer is a more selective antagonist of activin A and activin AB over activin B than is ActRIIA-Fc homodimer. In addition, the ActRIIA-Fc:ALK4-Fc heterodimer has substantially increased affinity for activin AC and greatly reduced affinity for BMP10 compared to ActRIIA-Fc homodimer. Accordingly, an ActRIIA-Fc:ALK4-Fc heterodimer will be more useful than ActRIIA-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to antagonize activin A and/or activin AB preferentially over activin B, and to obtain strong inhibition of activin AC, while avoiding inhibition of BMP10.

Example 16. Generation of a BMPRII-Fc:ALK1-Fc heterodimer

Applicants constructed a soluble BMPRII-Fc:ALK1-Fc heteromeric complex comprising the extracellular domains of human BMPRII and human ALK1, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as BMPRII-Fc fusion polypeptide and ALK1-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

Formation of heteromeric BMPRII-Fc:ALK1-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face.

The BMPRII-Fc polypeptide sequence (SEQ ID NO: 121) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51  HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101  TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151  GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201  EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251  KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG
301  FYPSDIAVEW ESNGQPENNY KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN
351  VFSCSVMHEA LHNHYTQKSL SLSPGK      (SEQ ID NO: 121)
```

The leader sequence and linker sequence are underlined. To promote formation of the BMPRII-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the BMPRII-Fc fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 121 may optionally be provided with the lysine removed from the C-terminus.

This BMPRII-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 122):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCGCAGAA TCAAGAACGC CTATGTGCGT
 101  TTAAAGATCC GTATCAGCAA GACCTTGGGA TAGGTGAGAG TAGAATCTCT
 151  CATGAAAATG GGACAATATT ATGCTCGAAA GGTAGCACCT GCTATGGCCT
 201  TTGGGAGAAA TCAAAAGGGG ACATAAATCT TGTAAAACAA GGATGTTGGT
 251  CTCACATTGG AGATCCCCAA GAGTGTCACT ATGAAGAATG TGTAGTAACT
 301  ACCACTCCTC CCTCAATTCA GAATGGAACA TACCGTTTCT GCTGTTGTAG
 351  CACAGATTTA TGTAATGTCA ACTTTACTGA GAATTTTCCA CCTCCTGACA
 401  CAACACCACT CAGTCCACCT CATTCATTTA ACCGAGATGA GACCGGTGGT
 451  GGAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC
 501  GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC
 551  GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT
 601  GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA
 651  GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG
 701  TCCTCACCGT CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC
 751  AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA
 801  AGCCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC
```

```
 851  GGAAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
 901  TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA
 951  GAACAACTAC AAGACCACGC CTCCCGTGCT GAAGTCCGAC GGCTCCTTCT
1001  TCCTCTATAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
1051  GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA
1101  GAAGAGCCTC TCCCTGTCTC CGGGTAAA    (SEQ ID NO: 122)
```

The mature BMPRII-Fc fusion polypeptide (SEQ ID NO: 123) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
   1  SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
  51  INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
 101  FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
 151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
 201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
 251  PQVYTLPPSR KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
 301  PVLKSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
 351  GK       (SEQ ID NO: 123)
```

The complementary form of ALK1-Fc fusion polypeptide (SEQ ID NO: 124) is as follows:

```
   1  MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
  51  GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
 101  NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
 151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
 201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
 251  PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP
 301  PVLDSDGSFF LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
 351  G        (SEQ ID NO: 124)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 121 and 123 above, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK1-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 124 may optionally be provided with a lysine added at the C-terminus.

This ALK1-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 125):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGACCCTGT GAAGCCGTCT CGGGGCCCGC
 101  TGGTGACCTG CACGTGTGAG AGCCCACATT GCAAGGGGCC TACCTGCCGG
 151  GGGGCCTGGT GCACAGTAGT GCTGGTGCGG GAGGAGGGGA GGCACCCCCA
 201  GGAACATCGG GGCTGCGGGA ACTTGCACAG GGAGCTCTGC AGGGGCCGCC
 251  CCACCGAGTT CGTCAACCAC TACTGCTGCG ACAGCCACCT CTGCAACCAC
 301  AACGTGTCCC TGGTGCTGGA GGCCACCCAA CCTCCTTCGG AGCAGCCGGG
 351  AACAGATGGC CAGCTGGCCA CCGGTGGTGG AACTCACACA TGCCCACCGT
 401  GCCCAGCACC TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA
 451  AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT
 501  GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG
 551  TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG
 601  TACAACAGCA CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA
 651  CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC
 701  CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA
 751  CCACAGGTGT ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA
 801  GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG
 851  TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACGA CACCACGCCT
 901  CCCGTGCTGG ACTCCGACGG CTCCTTCTTC CTCTATAGCG ACCTCACCGT
 951  GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC
1001  ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
1051  GGT          (SEQ ID NO: 125)
```

The mature ALK1-Fc fusion protein sequence (SEQ ID NO: 126) is as follows and may optionally be provided with a lysine added at the C-terminus.

```
   1  DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
  51  LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
 101  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 151  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 201  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV
 251  KGFYPSDIAV EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ
 301  GNVFSCSVMH EALHNHYTQK SLSLSPG          (SEQ ID NO: 126)
```

The BMPRII-Fc and ALK1-Fc proteins of SEQ ID NO: 123 and SEQ ID NO: 126, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK1-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond.

The BMPRII-Fc polypeptide sequence (SEQ ID NO: 411) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51  HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101  TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151  GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201  EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251  KVSNKALPAP IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG
301  FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
351  VFSCSVMHEA LHNHYTQKSL SLSPGK      (SEQ ID NO: 411)
```

The leader sequence and linker sequence are underlined. To promote formation of the BMPRII-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 411 may optionally be provided with the lysine removed from the C-terminus.

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

```
  1  SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
 51  INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
101  FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251  PQVYTLPPCR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP
301  PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  GK      (SEQ ID NO: 412)
```

The complementary form of ALK1-Fc fusion polypeptide (SEQ ID NO: 413) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
 51  GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
101  NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251  PQVCTLPPSR EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP
301  PVLDSDGSFF LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  GK       (SEQ ID NO: 413)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 411 and 412 above, four amino acid substitutions can be introduced into the Fc domain of the ALK1 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 413 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK1-Fc fusion protein sequence (SEQ ID NO: 414) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
 51  LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
101  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
151  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
201  KCKVSNKALP APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV
251  KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ
301  GNVFSCSVMH EALHNHYTQK SLSLSPGK       (SEQ ID NO: 414)
```

The BMPRII-Fc and ALK1-Fc proteins of SEQ ID NO: 412 and SEQ ID NO: 414, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK1-Fc.

Purification of various BMPRII-Fc:ALK1-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange

chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 17. Ligand binding profile of BMPRII-Fc:ALK1-Fc heterodimer compared to BMPRII-Fc homodimer and ALK1-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the BMPRII-Fc:ALK1-Fc heterodimeric complex described above with that of BMPRII-Fc and ALK1-Fc homodimeric complexes. The BMPRII-Fc:ALK1-Fc heterodimer, BMPRII-Fc homodimer, and ALK1-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of BMPRII-Fc:ALK1-Fc heterodimer compared to BMPRII-Fc homodimer and ALK1-Fc homodimer									
Ligand	BMPRII-Fc homodimer			ALK1-Fc homodimer			BMPRII-Fc:ALK1-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
BMP9	1.2×10^7	2.6×10^{-2}	2100	7.8×10^6	1.3×10^{-4}	16	1.2×10^6	4.1×10^{-4}	360
BMP10	2.6×10^7	2.5×10^{-3}	100	4.1×10^6	1.6×10^{-4}	38	1.5×10^7	3.5×10^{-4}	23
BMP15	9.9×10^6	2.8×10^{-3}	290	No binding			1.2×10^7	4.2×10^{-2}	3500

These comparative binding data demonstrate that the BMPRII-Fc:ALK1-Fc heterodimer has a binding profile/selectivity which differs from that of BMPRII-Fc homodimer but is similar to that of ALK1-Fc homodimer. For example, the BMPRII-Fc:ALK1-Fc heterodimer largely retains the strong binding to BMP9 and BMP10 characteristic of ALK1-Fc homodimer; however, the heterodimer displays modest selectivity for BMP10 over BMP9 not present with the homodimer. Also unlike ALK1-Fc homodimer, the BMPRII-Fc:ALK1-Fc heterodimer binds to BMP15, albeit with an affinity approximately an order of magnitude weaker than that of BMPRII-Fc homodimer. See **Figure 11**. Accordingly, a BMPRII-Fc:ALK1-Fc heterodimer will be unexpectedly useful in certain therapeutic applications where selective antagonism of BMP9 and particularly BMP10 is

advantageous, e.g., for inhibition of angiogenesis, or in applications where antagonism of BMP15 is also advantageous.

Example 18. Generation of a BMPRII-Fc:ALK2-Fc heterodimer

Applicants constructed a soluble BMPRII-Fc:ALK2-Fc heteromeric complex comprising the extracellular domains of human BMPRII and human ALK2, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as BMPRII-Fc fusion polypeptide and ALK2-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric BMPRII-Fc:ALK2-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the BMPRII-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 16 as SEQ ID NOs: 121-123. To promote formation of the BMPRII-Fc:ALK2-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the BMPRII-Fc fusion protein as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 121 and 123 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK2-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 9 as SEQ ID NOs: 136-138. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 121 and 123, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK2-Fc fusion polypeptide as indicated in Example 9. The amino acid sequences of SEQ ID NOs: 136 and 138 may optionally be provided with a lysine added at the C-terminus.

The BMPRII-Fc and ALK2-Fc fusion polypeptides of SEQ ID NO: 123 and SEQ ID NO: 138, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK2-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. BMPRII-Fc fusion polypeptide sequences (SEQ ID NOs: 411-412) are discussed in Example 16. To promote formation of the BMPRII-Fc:ALK2-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the BMPRII-Fc polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 411 and 412 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK2-Fc fusion polypeptide (SEQ ID NOs: 421-422) are discussed in Example 9. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 411-412, four amino acid substitutions can be introduced into the Fc domain of the ALK2 fusion polypeptide as indicated in Example 9. The amino acid sequences of SEQ ID NOs: 421-422 may optionally be provided with the lysine removed from the C-terminus.

The BMPRII-Fc and ALK2-Fc fusion polypeptides of SEQ ID NO: 412 and SEQ ID NO: 422, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK2-Fc.

Purification of various BMPRII-Fc:ALK2-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 19. Ligand binding profile of BMPRII-Fc:ALK2-Fc heterodimer compared to BMPRII-Fc homodimer and ALK2-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the BMPRII-Fc:ALK2-Fc heterodimeric complex described above with that of BMPRII-Fc and ALK2-Fc homodimeric complexes. The BMPRII-Fc:ALK2-Fc heterodimer, BMPRII-Fc homodimer, and ALK2-Fc homodimer were independently captured onto the system using an

anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of BMPRII-Fc:ALK2-Fc heterodimer compared to BMPRII-Fc homodimer and ALK2-Fc homodimer

Ligand	BMPRII-Fc homodimer			ALK2-Fc homodimer			BMPRII-Fc:ALK2-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin B	1.9×10^6	4.9×10^{-3}	2600	No binding			5.9×10^5	3.1×10^{-3}	5200
BMP5	1.9×10^6	1.9×10^{-2}	9900	No binding			1.8×10^6	5.0×10^{-3}	2800
BMP7	Transient*		> 93000	No binding			1.5×10^7	1.2×10^{-2}	760
BMP9	4.5×10^7	7.3×10^{-2}	1600	No binding			1.0×10^7	5.1×10^{-3}	500
BMP10	3.8×10^7	5.0×10^{-3}	130	No binding			1.1×10^8	3.4×10^{-2}	300
BMP15	5.8×10^6	4.2×10^{-3}	720	No binding			9.6×10^6	1.1×10^{-2}	1100

* Indeterminate due to transient nature of interaction

Example 20. Generation of a BMPRII-Fc:ALK3-Fc heterodimer

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

Formation of heteromeric BMPRII-Fc:ALK3-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the BMPRII-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 16 as SEQ ID NOs: 121-123. To promote formation of the BMPRII-Fc:ALK3-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid

substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the BMPRII-Fc fusion protein as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 121 and 123 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK3-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 4 as SEQ ID NOs: 115-117. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 121 and 123, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK3-Fc fusion polypeptide as indicated in Example 4. The amino acid sequences of SEQ ID NOs: 115 and 117 may optionally be provided with a lysine added at the C-terminus.

The BMPRII-Fc and ALK3-Fc fusion polypeptides of SEQ ID NO: 123 and SEQ ID NO: 117, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK3-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. BMPRII-Fc fusion polypeptide sequences (SEQ ID NOs: 411-412) are discussed in Example 16. To promote formation of the BMPRII-Fc:ALK3-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the BMPRII-Fc polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 411 and 412 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK3-Fc fusion polypeptide (SEQ ID NOs: 407-408) are discussed in Example 4. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 411-412, four amino acid substitutions can be introduced into the Fc domain of the ALK3 fusion polypeptide as indicated in Example 4. The amino acid sequences of SEQ ID NOs: 407 and 408 may optionally be provided with the lysine removed from the C-terminus.

The BMPRII-Fc and ALK3-Fc fusion polypeptides of SEQ ID NO: 412 and SEQ ID NO: 408, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK3-Fc.

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

Example 21. Ligand binding profile of BMPRII-Fc:ALK3-Fc heterodimer compared to BMPRII-Fc homodimer and ALK3-Fc homodimer

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

Ligand binding profile of BMPRII-Fc:ALK3-Fc heterodimer compared to BMPRII-Fc homodimer and ALK3-Fc homodimer									
Ligand	BMPRII-Fc homodimer			ALK3-Fc homodimer			BMPRII-Fc:ALK3-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin B	2.0×10^7	7.5×10^{-2}	3800	No binding			Minimal binding		
BMP2	Transient*		>2×10^6	6.2×10^5	1.4×10^{-4}	230	2.9×10^6	1.5×10^{-4}	51
BMP4	---			2.6×10^5	5.5×10^{-5}	210	9.1×10^5	9.1×10^{-5}	100
BMP5	---			2.9×10^4	2.3×10^{-3}	70000	4.3×10^5	1.4×10^{-3}	3200
BMP6	Transient*		>8900	1.4×10^5	4.9×10^{-3}	35000	3.6×10^5	5.9×10^{-4}	1600
BMP7	Transient*		>38000	1.2×10^6	1.8×10^{-2}	15000	1.2×10^7	1.2×10^{-2}	1000
BMP9	1.2×10^7	2.6×10^{-2}	2100	No binding			No binding		
BMP10	2.6×10^7	2.5×10^{-3}	100	---			6.8×10^5	1.6×10^{-3}	2400
BMP15	9.9×10^6	2.8×10^{-3}	290	---			9.1×10^5	5.5×10^{-3}	6000
GDF5	No binding			4.3×10^5	1.1×10^{-2}	22000	Minimal binding		

GDF6	Transient*	>88000	3.4×10^{4}	1.3×10^{-3}	40000	1.4×10^{6}	1.9×10^{-3}	1400
* Indeterminate due to transient nature of interaction --- Not tested								

These comparative binding data demonstrate that the BMPRII-Fc:ALK3-Fc heterodimer has ligand binding selectivity which is clearly unlike that of BMPRII-Fc homodimer but also differs from that of ALK3-Fc homodimer. BMPRII-Fc:ALK3-Fc heterodimer binds much more strongly to BMP6 than does ALK3-Fc homodimer, reflecting an off-rate nearly ten-fold slower. With its largely unchanged binding to BMP2 and BMP4, the BMPRII-Fc:ALK3 heterodimer can therefore be considered a joint inhibitor of BMP2, BMP4, and BMP6. This binding profile contrasts with that of ALK3-Fc homodimer, whose exceptionally strongly binding to BMP4 and BMP2 identifies it as highly selective for this ligand pair compared to four ligands with intermediate-level binding, including BMP6. See **Figure 12**. Accordingly, a BMPRII-Fc:ALK3-Fc heterodimer will be unexpectedly useful in certain therapeutic applications where joint antagonism of BMP2, BMP4, and BMP6 is advantageous.

Example 22. Generation of a BMPRII-Fc:ALK4-Fc heterodimer

Applicants constructed a soluble BMPRII-Fc:ALK4-Fc heteromeric complex comprising the extracellular domains of human BMPRII and human ALK4, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as BMPRII-Fc fusion polypeptide and ALK4-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric BMPRII-Fc:ALK4-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the BMPRII-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 16 as SEQ ID NOs: 121-123. To promote formation of the BMPRII-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the BMPRII-Fc fusion protein as indicated in Example 16. The amino acid sequences of

SEQ ID NOs: 121 and 123 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK4-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 1 as SEQ ID NOs: 104-106. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 121 and 123, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK4-Fc fusion polypeptide as indicated in Example 1. The amino acid sequences of SEQ ID NOs: 104 and 106 may optionally be provided with a lysine added at the C-terminus.

The BMPRII-Fc and ALK4-Fc fusion polypeptides of SEQ ID NO: 123 and SEQ ID NO: 106, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK4-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. BMPRII-Fc fusion polypeptide sequences (SEQ ID NOs: 411 and 412) are discussed in Example 16. To promote formation of the BMPRII-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the BMPRII-Fc polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 411 and 412 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK4-Fc fusion polypeptide (SEQ ID NOs: 403 and 404) are discussed in Example 1. To guide heterodimer formation with the BMPRII-Fc fusion polypeptide of SEQ ID NOs: 411 and 412, four amino acid substitutions can be introduced into the Fc domain of the ALK4 fusion polypeptide as indicated in Example 1. The amino acid sequences of SEQ ID NOs: 403 and 404 may optionally be provided with the lysine removed from the C-terminus.

The BMPRII-Fc and ALK4-Fc fusion polypeptides of SEQ ID NO: 412 and SEQ ID NO: 404, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising BMPRII-Fc:ALK4-Fc.

Purification of various BMPRII-Fc:ALK4-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the

following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

5

Example 23. Ligand binding profile of BMPRII-Fc:ALK4-Fc heterodimer compared to BMPRII-Fc homodimer and ALK4-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the BMPRII-Fc:ALK4-Fc heterodimeric complex described above with that of BMPRII-Fc and ALK4-Fc homodimeric complexes. The BMPRII-Fc:ALK4-Fc heterodimer, BMPRII-Fc homodimer, and ALK4-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

10

Ligand binding profile of BMPRII-Fc:ALK4-Fc heterodimer compared to BMPRII-Fc homodimer and ALK4-Fc homodimer

Ligand	BMPRII-Fc homodimer			ALK4-Fc homodimer			BMPRII-Fc:ALK4-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	Transient*		>43000	5.8×10^5	1.2×10^{-2}	20000	2.0×10^6	2.2×10^{-3}	1100
Activin B	2.0×10^7	7.5×10^{-2}	3800	No binding			1.6×10^6	2.6×10^{-3}	1700
Activin AB	---	---	---	4.4×10^6	6.4×10^{-3}	1500	3.6×10^6	5.0×10^{-4}	140
BMP9	1.2×10^7	2.6×10^{-2}	2100	No binding			Transient*		>140000
BMP10	2.6×10^7	2.5×10^{-3}	100	No binding			8.0×10^5	1.8×10^{-3}	2200
BMP15	9.9×10^6	2.8×10^{-3}	290	No binding			2.8×10^7	4.8×10^{-2}	1700

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

15 These comparative binding data demonstrate that the BMPRII-Fc:ALK4-Fc heterodimer has ligand binding selectivity which is unlike that of either BMPRII-Fc homodimer or ALK4-Fc homodimer. BMPRII-Fc:ALK4-Fc heterodimer differs from both homodimers by binding several activin ligands with high or intermediate strength and differs

from BMPRII-Fc homodimer by binding BMP15 only weakly. Most notably, BMPRII-Fc:ALK4-Fc heterodimer binds strongly and preferentially to the heterodimeric ligand activin AB. See **Figure 13**. Accordingly, a BMPRII-Fc:ALK4-Fc heterodimer will be unexpectedly useful in certain therapeutic applications where antagonism of activin A, activin B, and particularly activin AB are advantageous and where antagonism of BMP15 (which is heavily implicated in ovulation) is to be avoided.

Example 24. Generation of a TGFβRII-Fc:ALK1-Fc heterodimer

Applicants constructed a soluble TGFβRII-Fc:ALK1-Fc heteromeric complex comprising the extracellular domains of the short (canonical) isoform of human TGFβRII and human ALK1, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as $TGF\beta RII_{SHORT}$-Fc fusion polypeptide and ALK1-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric $TGF\beta RII_{SHORT}$-Fc:ALK1-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face.

The $TGF\beta RII_{SHORT}$-Fc polypeptide sequence (SEQ ID NO: 127) is shown below:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151 FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201 EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251 VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRKE
301 MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LKSDGSFFLY
351 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
    (SEQ ID NO: 127)
```

The leader sequence and linker sequence are underlined. To promote formation of the $TGF\beta RII_{SHORT}$-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be

introduced into the Fc domain of the TGFβRII$_{SHORT}$-Fc fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 127 may optionally be provided with the lysine removed from the C-terminus.

This TGFβRII$_{SHORT}$-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 128):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151  CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201  ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251  AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301  ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351  TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401  CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451  TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAACTCA
 501  CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT
 551  TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
 601  GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA
 651  GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC
 701  CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC
 751  GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC
 801  CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG
 851  GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGAAGGAG
 901  ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC
 951  CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT
1001  ACAAGACCAC GCCTCCCGTG CTGAAGTCCG ACGGCTCCTT CTTCCTCTAT
1051  AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC
1101  ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC
1151  TCTCCCTGTC TCCGGGTAAA                    (SEQ ID NO: 128)
```

The mature TGFβRII$_{SHORT}$-Fc fusion polypeptide (SEQ ID NO: 129) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
   1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
  51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
```

```
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GTHTCPPCPA
151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251  IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW
301  ESNGQPENNY KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351  LHNHYTQKSL SLSPGK      (SEQ ID NO: 129)
```

The polypeptide sequence of the complementary ALK1-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 16 as SEQ ID NOs: 124-126. To guide heterodimer formation with the TGFβRII$_{SHORT}$-Fc fusion polypeptide of SEQ ID NOs: 127 and 129, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK1-Fc fusion polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 124 and 126 may optionally be provided with a lysine added at the C-terminus.

The TGFβRII$_{SHORT}$-Fc and ALK1-Fc proteins of SEQ ID NO: 129 and SEQ ID NO: 126, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{SHORT}$-Fc:ALK1-Fc.

A variant TGFβRII-Fc:ALK1-Fc heteromeric complex may be generated in which the ALK1-Fc polypeptide described above (SEQ ID NO: 126) is paired with an Fc fusion protein comprising the extracellular domain of the long (A) isoform of TGFβRII (TGFβRII$_{LONG}$) in place of the extracellular domain of the short isoform.

The TGFβRII$_{LONG}$-Fc polypeptide sequence (SEQ ID NO: 130) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51  RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
101  TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151  KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201  ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251  EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301  EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ VSLTCLVKGF YPSDIAVEWE
351  SNGQPENNYK TTPPVLKSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
401  HNHYTQKSLS LSPGK   (SEQ ID NO: 130)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{LONG}$-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric

complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the TGFβRII$_{LONG}$-Fc fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 130 may optionally be provided with the lysine removed from the C-terminus.

This TGFβRII$_{LONG}$-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 131):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  ATGTGGAAAT GGAGGCCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
 151  AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
 201  CAACAACGGT GCAGTCAAGT TTCCACAACT GTGTAAATTT TGTGATGTGA
 251  GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
 301  ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
 351  GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCCAAGCTCC
 401  CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
 451  AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
 501  TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
 551  ATCCTGACAC CGCTCGTCGA ACTCACACAT GCCCACCGTG CCCAGCACCT
 601  GAACTCCTGG GGGGACCGTC AGTCTTCCTC TTCCCCCCAA AACCCAAGGA
 651  CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG
 701  TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG
 751  GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC
 801  GTACCGTGTG GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG
 851  GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC
 901  GAGAAAACCA TCTCCAAAGC CAAAGGGCAG CCCCGAGAAC CACAGGTGTA
 951  CACCCTGCCC CCATCCCGGA AGGAGATGAC CAAGAACCAG GTCAGCCTGA
1001  CCTGCCTGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT GGAGTGGGAG
1051  AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGAA
1101  GTCCGACGGC TCCTTCTTCC TCTATAGCAA GCTCACCGTG GACAAGAGCA
1151  GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG
1201  CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAA
      (SEQ ID NO: 131)
```

The mature TGFβRII$_{LONG}$-Fc fusion polypeptide (SEQ ID NO: 132) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRK
301  EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLKSDGSFFL
351  YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
     (SEQ ID NO: 132)
```

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond.

The TGFβRII$_{SHORT}$-Fc polypeptide sequence (SEQ ID NO: 415) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201  EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251  VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPCREE
301  MTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
351  SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
     (SEQ ID NO: 415)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{SHORT}$-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 415 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{SHORT}$-Fc fusion polypeptide (SEQ ID NO: 416) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GTHTCPPCPA
151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251  IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW
301  ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351  LHNHYTQKSL SLSPGK          (SEQ ID NO: 416)
```

Polypeptide sequences of the complementary ALK1-Fc fusion polypeptide (SEQ ID NOs: 413 and 414) are discussed in Example 16. To guide heterodimer formation with the $TGF\beta RII_{SHORT}$-Fc fusion polypeptide of SEQ ID NOs: 415 and 416, four amino acid substitutions can be introduced into the Fc domain of the ALK1 fusion polypeptide as indicated in Example 16. The amino acid sequences of SEQ ID NOs: 413 and 414 may optionally be provided with the lysine removed from the C-terminus.

The $TGF\beta RII_{SHORT}$-Fc and ALK1-Fc proteins of SEQ ID NO: 416 and SEQ ID NO: 414, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII-Fc:ALK1-Fc.

A variant TGFβRII-Fc:ALK1-Fc heteromeric complex may be generated in which the ALK1-Fc polypeptide described above (SEQ ID NO: 414) is paired with an Fc fusion protein comprising the extracellular domain of the long (A) isoform of TGFβRII ($TGF\beta RII_{LONG}$) in place of the extracellular domain of the short isoform.

The $TGF\beta RII_{LONG}$-Fc polypeptide sequence (SEQ ID NO: 417) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51  RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
101  TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151  KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201  ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251  EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301  EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF YPSDIAVEWE
351  SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
401  HNHYTQKSLS LSPGK   (SEQ ID NO: 417)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{LONG}$-Fc:ALK1-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 417 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{LONG}$-Fc fusion polypeptide (SEQ ID NO: 418) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPCRE
301  EMTKNQVSLW CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
351  YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
     (SEQ ID NO: 418)
```

The TGFβRII$_{LONG}$-Fc and ALK1-Fc proteins of SEQ ID NO: 418 and SEQ ID NO: 414, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{LONG}$-Fc:ALK1-Fc.

Purification of various TGFβRII-Fc:ALK1-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 25. Ligand binding profile of TGFβRII-Fc:ALK1-Fc heterodimer compared to TGFβRII-Fc homodimer and ALK1-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the TGFβRII$_{SHORT}$-Fc:ALK1-Fc heterodimeric complex described above with that of TGFβRII$_{SHORT}$-Fc and ALK1-Fc homodimeric complexes. The TGFβRII$_{SHORT}$-Fc:ALK1-Fc

heterodimer, TGFβRII$_{SHORT}$-Fc homodimer, and ALK1-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of TGFBRII$_{SHORT}$-Fc:ALK1-Fc heterodimer compared to TGFBRII$_{SHORT}$-Fc homodimer and ALK1-Fc homodimer									
Ligand	TGFBRII$_{SHORT}$-Fc homodimer			ALK1-Fc homodimer			TGFBRII$_{SHORT}$-Fc:ALK1-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
BMP9	No binding			7.9×10^6	1.3×10^{-4}	16	2.1×10^7	2.2×10^{-3}	110
BMP10	No binding			1.7×10^7	1.1×10^{-4}	6	1.2×10^7	9.6×10^{-4}	78
TGFβ1	4.2×10^7	1.1×10^{-3}	25	No binding			Transient*		>5300
TGFβ2	Transient*		>44000	No binding			No binding		
TGFβ3	5.9×10^7	5.9×10^{-3}	99	No binding			Transient*		>4700
* Indeterminate due to transient nature of interaction									

Example 26. Generation of a TGFβRII$_{SHORT}$-Fc:ALK5-Fc heterodimer

Applicants constructed a soluble TGFβRII$_{SHORT}$-Fc:ALK5-Fc heteromeric complex comprising the extracellular domains of the human TGFβRII short (canonical) isoform and human ALK5, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as TGFβRII$_{SHORT}$-Fc fusion polypeptide and ALK5-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric TGFβRII$_{SHORT}$-Fc:ALK5-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the TGFβRII$_{SHORT}$-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 24 as SEQ ID NOs: 127-129. To promote formation of the TGFβRII$_{SHORT}$-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two

amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the TGFβRII$_{SHORT}$-Fc fusion protein as indicated in Example 24. The amino acid sequences of SEQ ID NOs: 127 and 129 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK5-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 11 as SEQ ID NOs: 139-141. To guide heterodimer formation with the TGFβRII$_{SHORT}$-Fc fusion polypeptide of SEQ ID NOs: 127 and 129, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK5-Fc fusion polypeptide as indicated in Example 11. The amino acid sequences of SEQ ID NOs: 139 and 141 may optionally be provided with a lysine added at the C-terminus.

The TGFβRII$_{SHORT}$-Fc and ALK5-Fc fusion polypeptides of SEQ ID NO: 129 and SEQ ID NO: 141, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{SHORT}$-Fc:ALK5-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. TGFβRII$_{SHORT}$-Fc fusion polypeptide sequences (SEQ ID NOs: 415-416) are discussed in Example 24. To promote formation of the TGFβRII$_{SHORT}$-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the TGFβRII$_{SHORT}$-Fc polypeptide as indicated in Example 24. The amino acid sequences of SEQ ID NOs: 415-416 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK5-Fc fusion polypeptide (SEQ ID NOs: 423-424) are discussed in Example 11. To guide heterodimer formation with the TGFβRII$_{SHORT}$-Fc fusion polypeptide of SEQ ID NOs: 415-416, four amino acid substitutions can be introduced into the Fc domain of the ALK5 fusion polypeptide as indicated in Example 11. The amino acid sequences of SEQ ID NOs: 423-424 may optionally be provided with the lysine removed from the C-terminus.

The TGFβRII$_{SHORT}$-Fc and ALK5-Fc fusion polypeptides of SEQ ID NO: 416 and SEQ ID NO: 424, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{SHORT}$-Fc:ALK5-Fc.

Purification of various TGFβRII$_{SHORT}$-Fc:ALK5-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 27. Generation of a TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer

Applicants constructed a soluble TGFβRII$_{LONG}$-Fc:ALK5-Fc heteromeric complex comprising the extracellular domain of the long (A) isoform of human TGFβRII and the extracellular domain of human ALK5, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as TGFβRII$_{LONG}$-Fc fusion polypeptide and ALK5-Fc fusion polypeptide, respectively, and the sequences for each are provided herein.

Formation of heteromeric TGFβRII$_{LONG}$-Fc:ALK5-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The polypeptide sequence of the TGFβRII$_{LONG}$-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided above in Example 24 as SEQ ID NOs: 130-132. To promote formation of the TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the TGFβRII$_{LONG}$-Fc fusion protein as indicated in Example 24. The amino acid sequences of SEQ ID NOs: 130 and 132 may optionally be provided with the lysine removed from the C-terminus.

The polypeptide sequence of the complementary ALK5-Fc fusion polypeptide and a nucleic acid sequence encoding it are provided in Example 11 as SEQ ID NOs: 139-141. To guide heterodimer formation with the TGFβRII$_{LONG}$-Fc fusion polypeptide of SEQ ID NOs: 130 and 132, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK5-Fc fusion polypeptide as indicated in Example 11. The amino acid sequences of SEQ ID NOs: 139 and 142 may optionally be provided with a lysine added at the C-terminus.

The TGFβRII$_{LONG}$-Fc and ALK5-Fc fusion polypeptides of SEQ ID NO: 132 and SEQ ID NO: 141, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{LONG}$-Fc:ALK5-Fc.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond. TGFβRII$_{LONG}$-Fc fusion polypeptide sequences (SEQ ID NOs: 417-418) are discussed in Example 24. To promote formation of the TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the TGFβRII$_{LONG}$-Fc polypeptide as indicated in Example 24. The amino acid sequences of SEQ ID NOs: 417-418 may optionally be provided with the lysine removed from the C-terminus.

Polypeptide sequences of the complementary ALK5-Fc fusion polypeptide (SEQ ID NOs: 423-424) are discussed in Example 11. To guide heterodimer formation with the TGFβRII$_{LONG}$-Fc fusion polypeptide of SEQ ID NOs: 417-418, four amino acid substitutions can be introduced into the Fc domain of the ALK5 fusion polypeptide as indicated in Example 11. The amino acid sequences of SEQ ID NOs: 423-424 may optionally be provided with the lysine removed from the C-terminus.

The TGFβRII$_{LONG}$-Fc and ALK5-Fc fusion polypeptides of SEQ ID NO: 418 and SEQ ID NO: 424, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising TGFβRII$_{LONG}$-Fc:ALK5-Fc.

Purification of various TGFβRII$_{LONG}$-Fc:ALK5-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 28. Activity profiles of TGFβRII-Fc:ALK5-Fc heterodimers compared to TGFβRII-Fc homodimer and ALK5-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the TGFβRII$_{SHORT}$-Fc:ALK5-Fc and TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimeric complexes described in Examples 26-27 with that of TGFβRII$_{SHORT}$-Fc and ALK5-Fc homodimeric complexes. The heteromeric or homomeric protein complexes were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profiles of TGFβRII-Fc:ALK5-Fc heterodimers compared to TGFβRII-Fc homodimer and ALK5-Fc homodimer

Ligand	ALK5-Fc Homodimer			TGFβRII$_{SHORT}$-Fc Homodimer			TGFβRII$_{SHORT}$-Fc:ALK5-Fc Heterodimer*			TGFβRII$_{LONG}$-Fc:ALK5-Fc Heterodimer*		
	k_a	k_d	K_D	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
TGFβ1	No binding			5.6×10^7	1.1×10^{-3}	20	1.4×10^8	1.7×10^{-3}	12	6.6×10^7	9.2×10^{-4}	14
TGFβ2	No binding			2.1×10^5	2.2×10^{-3}	11000	6.6×10^6	2.9×10^{-6}	0.4	4.2×10^6	2.8×10^{-7}	0.07
TGFβ3	No binding			1.9×10^7	1.4×10^{-3}	71	2.7×10^7	1.0×10^{-3}	38	2.7×10^7	1.0×10^{-3}	38

* Low signal which suggests that a substantial fraction of the protein is inactive

These comparative binding data indicate that the ligand binding profiles of TGFβRII-Fc:ALK5-Fc heterodimers are markedly different from that of TGFβRII-Fc homodimer and from ALK5-Fc homodimer, which did not bind any ligands. Based on the equilibrium dissociation constant (K_D), TGFβRII-Fc homodimer bound TGFβ1 and TGFβ3 with much higher affinity than TGFβ1, even though off-rates for the three TGFβ ligands were similar. In contrast, TGFβRII-Fc:ALK5-Fc heterodimers displayed high selectivity for TGFβ2 over TGFβ1/TGFβ3. In particular, TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer bound TGFβ2 with an affinity approximately five orders of magnitude higher and an off-rate approximately four orders of magnitude slower than did TGFβRII-Fc homodimer. TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer also bound TGFβ2 more strongly than did heterodimer containing the short isoform. See **Figure 14**. Neither of the TGFβRII-Fc:ALK5-Fc heterodimers was able to bind BMP9 or BMP10 (data not shown), which distinguishes these TGFβRII-Fc:ALK5-Fc heterodimers from TGFβRII-Fc:ALK1-Fc heterodimer (see Example 25). Sensograms for the two TGFβRII-Fc:ALK5-Fc heterodimers exhibited low signal amplitude which suggests that a substantial fraction of each protein was inactive.

To better interpret these data obtained by surface plasmon resonance, a reporter gene assay in A549 cells was used to determine the ability of TGFβRII fusion proteins to inhibit activity of TGFβ1, TGFβ2, and TGFβ3. This assay is based on a human lung carcinoma cell line transfected with reporter plasmids pGL3(CAGA)12-firefly luciferase (Dennler et al, 1998, EMBO 17: 3091-3100) and pRLCMV-renilla luciferase, the latter to control for transfection efficiency. The CAGA motif is present in the promoters of TGFβ-responsive genes (for example, PAI-1), so this vector is of general use for factors signaling through SMAD2 and SMAD3.

On the first day of the assay, A549 cells (ATCC®: CCL-185™) were distributed in 48-well plates at $6.5x10^4$ cells per well and incubated overnight. All incubations were at 37°C and 5% CO_2 in a tissue culture incubator unless otherwise indicated. On the second day, a solution containing 10 μg pGL3(CAGA)12-firefly luciferase, 100 ng pRLCMV-renilla luciferase, 30 μL X-tremeGENE 9 (Roche Applied Science), and 970 μL OptiMEM (Invitrogen) was preincubated for 30 min at room temperature, then added to 24 mL Eagle's minimum essential medium (EMEM, ATCC®) supplemented with 0.1% BSA. Medium was removed from the plated cells and this transfection mixture was applied to the cells (500 μl/well) for an overnight incubation. On the third day, medium was removed, and cells were incubated overnight with a mixture of ligands and inhibitors prepared as described below.

Serial dilutions of test articles were made in a 48-well plate in a 200 μL volume of assay butter (EMEM + 0.1 % BSA). An equal volume of assay buffer containing the test ligand was added to obtain a final ligand concentration equal to the EC_{50} determined previously. Human TGFβ1, TGFβ2, and TGFβ3 were obtained from PeproTech. Test solutions were incubated for 30 minutes, then 250 μL of the mixture was added to the transfected cells. Each concentration of test article was determined in duplicate. After incubation with test solutions overnight, cells were rinsed with phosphate-buffered saline, then lysed with passive lysis buffer (Promega E1941) and stored overnight at -70°C. On the fourth and final day, plates were warmed to room temperature with gentle shaking. Cell lysates were transferred to a chemiluminescence plate (96-well) and analyzed in a luminometer with reagents from a Dual-Luciferase Reporter Assay system (Promega E1980) to determine normalized luciferase activity.

This assay was used to compare the ability of TGFβRII fusion protein variants to inhibit cell signaling by TGFβRII ligands. Results are shown in the table below.

Inhibitory Activity of TGFβRII Fusion Proteins in A549 Cells			
Construct	IC_{50} (pM)		
	TGFβ1 (640 pg/mL)	TGFβ2 (480 pg/mL)	TGFβ3 (270 pg/mL)
$TGF\beta RII_{SHORT}$-Fc homodimer	90	---	9
$TGF\beta RII_{SHORT}$-Fc:ALK5-Fc heterodimer*	< 350*	~ 200*	< 90*
$TGF\beta RII_{LONG}$-Fc:ALK5-Fc heterodimer	204	154	35
--- No inhibition (tested at concentrations up to 10 nM) * Value imprecise due to range of concentrations tested			

Results with TGFβRII-Fc homodimer were consistent with previous reports concerning wild-type $TGF\beta RII_{SHORT}$-Fc and $TGF\beta RII_{LONG}$-Fc homodimers (del Re et al., J Biol Chem 279:22765, 2004). In this experiment, $TGF\beta RII_{SHORT}$-Fc homodimer potently inhibited TGFβ1 and TGFβ3 but was unable to inhibit TGFβ2 at homodimer concentrations up to 10 nM. This finding is consistent with the low affinity of TGFβ2 binding to TGFβRII-Fc homodimer but oddly inconsistent with its slow off-rate (see binding results above). In contrast, TGFβRII-Fc:ALK5-Fc heterodimers potently inhibited all three TGFβ ligands in a cellular environment. Accordingly, a TGFβRII-Fc:ALK5-Fc heterodimer will be unexpectedly useful in certain therapeutic applications where preferential antagonism of TGFβ2 – or combined antagonism of TGFβ1, TGFβ2, and TGFβ3 – are advantageous.

Example 29. Generation of MISRII-Fc:ALK3-Fc heterodimers

A soluble MISRII-Fc:ALK3-Fc heteromeric complex can be generated comprising the extracellular domains of human MISRII and human ALK3, which can each be separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as MISRII-Fc fusion polypeptide and ALK3-Fc fusion polypeptide, respectively.

Formation of heteromeric MISRII-Fc:ALK3-Fc may be guided by approaches similar to those described in Example 1. In a first approach, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face.

The MISRII-Fc polypeptide sequence (SEQ ID NO: 133) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
 51  DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101  PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151  SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
201  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ
301  VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLKSDG SFFLYSKLTV
351  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 133)
```

The leader sequence and linker sequence are underlined. To promote formation of the MISRII-Fc:ALK3-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the MISRII fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 133 may optionally be provided with the lysine removed from the C-terminus.

The mature MISRII-Fc fusion polypeptide (SEQ ID NO: 135) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  PPNRRTCVFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51  TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
101  ANYSHLPPPG SPGTPGSQGP QAAPGESIWM ALTGGGTHTC PPCPAPELLG
151  GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
201  AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
251  SKAKGQPREP QVYTLPPSRK EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ
301  PENNYKTTPP VLKSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
351  TQKSLSLSPG K        (SEQ ID NO: 135)
```

In this first approach, the polypeptide sequence of the complementary ALK3-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 4 as SEQ ID NOs: 115-117.

The MISRII-Fc and ALK3-Fc proteins of SEQ ID NO: 135 and SEQ ID NO: 117, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising MISRII-Fc:ALK3-Fc. Similar heteromeric complexes may generated by pairing the ALK3-Fc protein described above (SEQ ID NO: 117) with an Fc

fusion protein comprising the extracellular domain of MISRII isoform 2 or or the extracellular domain of MISRII isoform 3 in place of the extracellular domain of MISRII isoform 1.

In a second approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains can be altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond.

The MISRII-Fc polypeptide sequence (SEQ ID NO: 419) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
 51  DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101  PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151  SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
201  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ
301  VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
351  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
     (SEQ ID NO: 419)
```

The leader sequence and linker sequence are underlined. To promote formation of the MISRII-Fc:ALK3-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 419 may optionally be provided with the lysine removed from the C-terminus.

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

```
  1  PPNRRTCVFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51  TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
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 VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
351  TQKSLSLSPG K        (SEQ ID NO: 420)
```

In this second approach, the polypeptide sequence of the complementary ALK3-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 4 as SEQ ID NOs: 407-408.

The MISRII-Fc and ALK3-Fc proteins of SEQ ID NO: 420 and SEQ ID NO: 408, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising MISRII-Fc:ALK3-Fc. Similar heteromeric complexes may be generated by pairing the ALK3-Fc protein described above (SEQ ID NO: 408) with an Fc fusion protein comprising the extracellular domain of MISRII isoform 2 or the extracellular domain of MISRII isoform 3 in place of the extracellular domain of MISRII isoform 1 described above .

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

Example 30. Generation of an ActRIIA-Fc:ActRIIB-Fc heterodimer

A soluble ActRIIA-Fc:ActRIIB-Fc heteromeric complex can be generated comprising the extracellular domains of human ActRIIA and human ActRIIB, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIA-Fc and ActRIIB-Fc fusion proteins, respectively.

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

Electrostatic Approach

In a first approach, the polypeptide sequence of the ActRIIA-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 14 as SEQ ID NOs: 118-120.

The polypeptide sequence of the complementary ActRIIB-Fc fusion protein (SEQ ID NO: 153) employs the TPA leader and is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS

```
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
301  EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK          (SEQ ID NO: 153)
```

The leader and linker sequences are underlined. To promote formation of the ActRIIA-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 153 may optionally be provided with the lysine removed from the C-terminus.

The mature ActRIIB-Fc fusion protein sequence is as follows (SEQ ID NO: 154) and may optionally be provided with the lysine removed from the C-terminus.

```
  1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
251  REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYDTT PPVLDSDGSF
301  FLYSDLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
     (SEQ ID NO: 154)
```

The ActRIIA-Fc and ActRIIB-Fc fusion proteins of SEQ ID NO: 120 and SEQ ID NO: 154, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIA-Fc:ActRIIB-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are both linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, an alternative polypeptide sequence of the ActRIIA-Fc fusion protein (SEQ ID NO: 151) employs the TPA leader and is as follows.

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
```

```
 51  GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101  DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151  CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201  VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251  PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
301  VEWESNGQPE NNYDTTPPVL DSDGSFFLYS DLTVDKSRWQ QGNVFSCSVM
351  HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 151)
```

The leader and linker sequences are underlined. To promote formation of the ActRIIA-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 151 may optionally be provided with the lysine removed from the C-terminus.

The mature ActRIIA-Fc fusion protein sequence is as follows (SEQ ID NO: 152) and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51  IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101  EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151  SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201  SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
251  SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYDT TPPVLDSDGS
301  FFLYSDLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
     (SEQ ID NO: 152)
```

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

The ActRIIA-Fc and ActRIIB-Fc fusion proteins of SEQ ID NO: 152 and SEQ ID NO: 102, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ActRIIA-Fc:ActRIIB-Fc.

Hydrophobic Approach

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. The polypeptide

sequence of the ActRIIA-Fc fusion protein is provided in Example 14 as SEQ ID NOs: 409-410.

The complementary form of ActRIIB-Fc fusion polypeptide (SEQ ID NO: 453) is as follows:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101 ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251 APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV KGFYPSDIAV
301 EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQK SLSLSPGK          (SEQ ID NO: 453)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ActRIIA-Fc fusion polypeptide of SEQ ID NOs: 409-410, four amino acid substitutions can be introduced into the Fc domain of the ActRIIB-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 453 may optionally be provided with a lysine removed from the C-terminus.

The mature ActRIIB-Fc fusion protein sequence (SEQ ID NO: 454) is as follows and may optionally be provided with a lysine removed from the C-terminus.

```
  1 GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51 IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101 GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151 RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVCTLPPS
251 REEMTKNQVS LSCAVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301 FLVSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
    (SEQ ID NO: 454)
```

The ActRIIA-Fc and ActRIIB-Fc proteins of SEQ ID NO: 410 and SEQ ID NO: 454, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:ActRIIB-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, an alternative polypeptide sequence of the ActRIIA-Fc fusion protein (SEQ ID NO: 451) employs the TPA leader and is as follows.

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51  GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101  DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151  CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201  VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251  PAPIEKTISK AKGQPREPQV CTLPPSREEM TKNQVSLSCA VKGFYPSDIA
301  VEWESNGQPE NNYKTTPPVL DSDGSFFLVS KLTVDKSRWQ QGNVFSCSVM
351  HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 451)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 401-402, four amino acid substitutions can be introduced into the Fc domain of the ActRIIA-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 451 may optionally be provided with the lysine removed from the C-terminus.

The mature ActRIIA-Fc fusion protein sequence is as follows (SEQ ID NO: 452) and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51  IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101  EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151  SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201  SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVCTLPP
251  SREEMTKNQV SLSCAVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
301  FFLVSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
     (SEQ ID NO: 452)
```

The polypeptide sequence of the ActRIIB-Fc fusion protein is provided in Example 1 as SEQ ID NOs: 401-402.

The ActRIIA-Fc and ActRIIB-Fc proteins of SEQ ID NO: 452 and SEQ ID NO: 402, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:ActRIIB-Fc.

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

Example 31. Generation of an ActRIIA-Fc:BMPRII-Fc heterodimer

A soluble ActRIIA-Fc:BMPRII-Fc heteromeric complex can be generated comprising the extracellular domains of human ActRIIA and human BMPRII, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIA-Fc and BMPRII-Fc fusion proteins, respectively.

Formation of heteromeric ActRIIA-Fc:BMPRII-Fc may be guided by approaches similar to those described in Example 30.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ActRIIA-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 14 as SEQ ID NOs: 118-120.

The polypeptide sequence of the complementary BMPRII-Fc fusion protein (SEQ ID NO: 155) employs the TPA leader and is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51  HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101  TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151  GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201  EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251  KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG
301  FYPSDIAVEW ESNGQPENNY DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN
351  VFSCSVMHEA LHNHYTQKSL SLSPGK      (SEQ ID NO: 155)
```

The leader and linker sequences are underlined. To promote formation of the ActRIIA-Fc:BMPRII-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 155 may optionally be provided with the lysine removed from the C-terminus.

The mature BMPRII-Fc fusion protein sequence is as follows (SEQ ID NO: 156) and may optionally be provided with the lysine removed from the C-terminus.

```
  1  SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
 51  INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
101  FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251  PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP
301  PVLDSDGSFF LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  GK        (SEQ ID NO: 156)
```

The ActRIIA-Fc and BMPRII-Fc fusion proteins of SEQ ID NO: 120 and SEQ ID NO: 156, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIA-Fc:BMPRII-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, the alternative polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 30 as SEQ ID NOs: 151-152.

The polypeptide sequence of the BMPRII-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 16 as SEQ ID NOs: 121-123.

The ActRIIA-Fc and BMPRII-Fc fusion proteins of SEQ ID NO: 152 and SEQ ID NO: 123, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ActRIIA-Fc:BMPRII-Fc.

Hydrophobic Approach

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. The polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 14 as SEQ ID NOs 409-410.

The complementary form of BMPRII-Fc fusion polypeptide (SEQ ID NO: 455) is as follows:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51 HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101 TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151 GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201 EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251 KVSNKALPAP IEKTISKAKG QPREPQVCTL PPSREEMTKN QVSLSCAVKG
301 FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN
351 VFSCSVMHEA LHNHYTQKSL SLSPGK      (SEQ ID NO: 455)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ActRIIA-Fc fusion polypeptide of SEQ ID NOs: 409-410, four amino acid substitutions can be introduced into the Fc domain of the BMPRII-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 455 may optionally be provided with a lysine removed from the C-terminus.

The mature BMPRII-Fc fusion protein sequence (SEQ ID NO: 456) is as follows and may optionally be provided with a lysine removed from the C-terminus.

```
  1 SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
 51 INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
101 FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
151 KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201 YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251 PQVCTLPPSR EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP
301 PVLDSDGSFF LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351 GK      (SEQ ID NO: 456)
```

The ActRIIA-Fc and BMPRII-Fc proteins of SEQ ID NO: 410 and SEQ ID NO: 456, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:BMPRII-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 30 as SEQ ID NOs: 451-452.

The polypeptide sequence of the BMPRII-Fc fusion protein is provided in Example 16 as SEQ ID NOs: 411-412.

The ActRIIA-Fc and BMPRII-Fc proteins of SEQ ID NO: 452 and SEQ ID NO: 412, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:BMPRII-Fc.

Purification of various ActRIIA-Fc:BMPRII-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 32. Generation of an ActRIIA-Fc:MISRII-Fc heterodimer

A soluble ActRIIA-Fc:MISRII-Fc heteromeric complex can be generated comprising the extracellular domains of human ActRIIA and human MISRII, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIA-Fc and MISRII-Fc fusion proteins, respectively.

Formation of heteromeric ActRIIA-Fc:MISRII-Fc may be guided by approaches similar to those described in Example 30.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ActRIIA-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 14 as SEQ ID NOs: 118-120.

The polypeptide sequence of the complementary MISRII-Fc fusion protein (SEQ ID NO: 161) employs the TPA leader and is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
 51  DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101  PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151  SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
201  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ
301  VSLTCLVKGF YPSDIAVEWE SNGQPENNYD TTPPVLDSDG SFFLYSDLTV
351  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 161)
```

The leader and linker sequences are underlined. To promote formation of the ActRIIA-Fc:MISRII-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 161 may optionally be provided with the lysine removed from the C-terminus.

The mature MISRII-Fc fusion protein sequence is as follows (SEQ ID NO: 162) and may optionally be provided with the lysine removed from the C-terminus.

```
  1  PPNRRTCVFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51  TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
101  ANYSHLPPPG SPGTPGSQGP QAAPGESIWM ALTGGGTHTC PPCPAPELLG
151  GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
201  AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
251  SKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ
301  PENNYDTTPP VLDSDGSFFL YSDLTVDKSR WQQGNVFSCS VMHEALHNHY
351  TQKSLSLSPG K        (SEQ ID NO: 162)
```

The ActRIIA-Fc and MISRII-Fc fusion proteins of SEQ ID NO: 120 and SEQ ID NO: 162, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIA-Fc:MISRII-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, the alternative polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 30 as SEQ ID NOs: 151 and 152.

The polypeptide sequence of the MISRII-Fc fusion protein is provided in Example 29 as SEQ ID NOs: 133 and 135.

The ActRIIA-Fc and MISRII-Fc fusion proteins of SEQ ID NO: 152 and SEQ ID NO: 135, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ActRIIA-Fc:MISRII-Fc.

Hydrophobic Approach

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. The polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 14 as SEQ ID NOs 409-410.

The complementary form of MISRII-Fc fusion polypeptide (SEQ ID NO: 457) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
 51  DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101  PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151  SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
201  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSREEMTKNQ
301  VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSKLTV
351  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
     (SEQ ID NO: 457)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ActRIIA-Fc fusion polypeptide of SEQ ID NOs: 409-410, four amino acid substitutions can be introduced into the Fc domain of the MISRII-Fc fusion polypeptide as indicated by

double underline above. The amino acid sequence of SEQ ID NO: 457 may optionally be provided with a lysine removed from the C-terminus.

The mature MISRII-Fc fusion protein sequence (SEQ ID NO: 458) is as follows and may optionally be provided with a lysine removed from the C-terminus.

```
  1  PPNRRTCVFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51  TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
101  ANYSHLPPPG SPGTPGSQGP QAAPGESIWM ALTGGGTHTC PPCPAPELLG
151  GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
201  AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
251  SKAKGQPREP QVCTLPPSRE EMTKNQVSLS CAVKGFYPSD IAVEWESNGQ
301  PENNYKTTPP VLDSDGSFFL VSKLTVDKSR WQQGNVFSCS VMHEALHNHY
351  TQKSLSLSPG K        (SEQ ID NO: 458)
```

The ActRIIA-Fc and MISRII-Fc proteins of SEQ ID NO: 410 and SEQ ID NO: 458, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:MISRII-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 30 as SEQ ID NOs: 451-452.

The polypeptide sequence of the MISRII-Fc fusion protein is provided in Example 29 as SEQ ID NOs: 419-420.

The ActRIIA-Fc and MISRII-Fc proteins of SEQ ID NO: 452 and SEQ ID NO: 420, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:MISRII-Fc.

Purification of various ActRIIA-Fc:MISRII-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 33. Generation of an ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc heterodimer

A soluble ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc heteromeric complex can be generated comprising the extracellular domains of human ActRIIA and human TGFβRII$_{SHORT}$, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIA-Fc and TGFβRII$_{SHORT}$-Fc fusion proteins, respectively.

Formation of heteromeric ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc may be guided by approaches similar to those described in Example 30.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ActRIIA-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 14 as SEQ ID NOs: 118-120.

The polypeptide sequence of the complementary TGFβRII$_{SHORT}$-Fc fusion protein (SEQ ID NO: 157) employs the TPA leader and is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51  QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101  TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151  FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201  EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251  VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
301  MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYDTTPPV LDSDGSFFLY
351  SDLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
     (SEQ ID NO: 157)
```

The leader and linker sequences are underlined. To promote formation of the ActRIIA-Fc: TGFβRII$_{SHORT}$-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above.

The amino acid sequence of SEQ ID NO: 157 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{SHORT}$-Fc fusion protein sequence is as follows (SEQ ID NO: 158) and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101  MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GTHTCPPCPA
151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251  IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW
301  ESNGQPENNY DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA
351  LHNHYTQKSL SLSPGK      (SEQ ID NO: 158)
```

The ActRIIA-Fc and TGFβRII$_{SHORT}$-Fc fusion proteins of SEQ ID NO: 120 and SEQ ID NO: 158, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, the alternative polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 30 as SEQ ID NOs: 151-152.

The polypeptide sequence of the TGFβRII$_{SHORT}$-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 24 as SEQ ID NOs: 127-129.

The ActRIIA-Fc and TGFβRII$_{SHORT}$-Fc fusion proteins of SEQ ID NO: 152 and SEQ ID NO: 129, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc.

Hydrophobic Approach

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. The polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 14 as SEQ ID NOs 409-410.

The complementary form of TGFβRII$_{SHORT}$-Fc fusion polypeptide (SEQ ID NO: 459) is as follows:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151 FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201 EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251 VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VCTLPPSREE
301 MTKNQVSLSC AVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLV
351 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
    (SEQ ID NO: 459)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ActRIIA-Fc fusion polypeptide of SEQ ID NOs: 409-410, four amino acid substitutions can be introduced into the Fc domain of the TGFβRII$_{SHORT}$-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 459 may optionally be provided with a lysine removed from the C-terminus.

The mature TGFβRII$_{SHORT}$-Fc fusion protein sequence (SEQ ID NO: 460) is as follows and may optionally be provided with a lysine removed from the C-terminus.

```
  1 TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51 ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101 MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GTHTCPPCPA
151 PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201 VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251 IEKTISKAKG QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW
301 ESNGQPENNY KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA
351 LHNHYTQKSL SLSPGK          (SEQ ID NO: 460)
```

The ActRIIA-Fc and TGFβRII$_{SHORT}$-Fc proteins of SEQ ID NO: 410 and SEQ ID NO: 460, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 30 as SEQ ID NOs: 451-452.

The polypeptide sequence of the TGFβRII$_{SHORT}$-Fc fusion protein is provided in Example 24 as SEQ ID NOs: 415-416.

The ActRIIA-Fc and TGFβRII$_{SHORT}$-Fc proteins of SEQ ID NO: 452 and SEQ ID NO: 416, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc.

Purification of various ActRIIA-Fc:TGFβRII$_{SHORT}$-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 34. Generation of an ActRIIA-Fc:TGFβRII$_{LONG}$-Fc heterodimer

A soluble ActRIIA-Fc:TGFβRII$_{LONG}$-Fc heteromeric complex can be generated comprising the extracellular domains of human ActRIIA and human TGFβRII$_{LONG}$, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIA-Fc and TGFβRII$_{LONG}$-Fc fusion proteins, respectively.

Formation of heteromeric ActRIIA-Fc:TGFβRII$_{LONG}$-Fc may be guided by approaches similar to those described in Example 30.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ActRIIA-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 14 as SEQ ID NOs: 118-120.

The polypeptide sequence of the complementary TGFβRII$_{LONG}$-Fc fusion protein (SEQ ID NO: 159) employs the TPA leader and is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51  RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
101  TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151  KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201  ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251  EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301  EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE
351  SNGQPENNYD TTPPVLDSDG SFFLYSDLTV DKSRWQQGNV FSCSVMHEAL
401  HNHYTQKSLS LSPGK      (SEQ ID NO: 159)
```

The leader and linker sequences are underlined. To promote formation of the ActRIIA-Fc:TGFβRII$_{LONG}$-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 159 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{LONG}$-Fc fusion protein sequence is as follows (SEQ ID NO: 160) and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE
301  EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYDTTPP VLDSDGSFFL
351  YSDLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
     (SEQ ID NO: 160)
```

The ActRIIA-Fc and TGFβRII$_{LONG}$-Fc fusion proteins of SEQ ID NO: 120 and SEQ ID NO: 160, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIA-Fc:TGFβRII$_{LONG}$-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this

example, the alternative polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 30 as SEQ ID NOs: 151-152.

The polypeptide sequence of the TGFβRII$_{LONG}$-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 24 as SEQ ID NOs: 130-132.

The ActRIIA-Fc and TGFβRII$_{LONG}$-Fc fusion proteins of SEQ ID NO: 152 and SEQ ID NO: 132, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ActRIIA-Fc:TGFβRII$_{LONG}$-Fc.

Hydrophobic Approach

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. The polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 14 as SEQ ID NOs 409-410.

The complementary form of TGFβRII$_{LONG}$-Fc fusion polypeptide (SEQ ID NO: 461) is as follows:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51 RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
101 TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151 KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301 EKTISKAKGQ PREPQVCTLP PSREEMTKNQ VSLSCAVKGF YPSDIAVEWE
351 SNGQPENNYK TTPPVLDSDG SFFLVSKLTV DKSRWQQGNV FSCSVMHEAL
401 HNHYTQKSLS LSPGK   (SEQ ID NO: 461)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ActRIIA-Fc fusion polypeptide of SEQ ID NOs: 409-410, four amino acid substitutions can be introduced into the Fc domain of the ActRIIB-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 461 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{LONG}$-Fc fusion protein sequence (SEQ ID NO: 462) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVCTLPPSRE
301  EMTKNQVSLS CAVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
351  VSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
     (SEQ ID NO: 462)
```

The ActRIIA-Fc and TGFβRII$_{LONG}$-Fc proteins of SEQ ID NO: 410 and SEQ ID NO: 462, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc: TGFβRII$_{LONG}$-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ActRIIA-Fc fusion protein is provided in Example 30 as SEQ ID NOs: 451-452.

The polypeptide sequence of the TGFβRII$_{LONG}$-Fc fusion protein is provided in Example 24 as SEQ ID NOs: 417-418.

The ActRIIA-Fc and TGFβRII$_{LONG}$-Fc proteins of SEQ ID NO: 452 and SEQ ID NO: 418, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ActRIIA-Fc:TGFβRII$_{LONG}$-Fc.

Purification of various ActRIIA-Fc:TGFβRII$_{LONG}$-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 35. Generation of additional heterodimers comprising two type II receptors

Formation of additional heterdimeric protein complexes comprising extracellular domains of two type II receptors may be guided by approaches similar to those described in Example 30. Amino acid SEQ ID NOs for these variant heterodimers are disclosed in the following table, which for completeness also includes the heterodimers already discussed that comprise two type II receptors (Examples 30-34). As disclosed in Examples 30-34, the C-terminal lysine of each receptor-Fc fusion protein may optionally be included or omitted.

In each case, a soluble heteromeric complex can be generated comprising the extracellular domains (ECD) of two different type II receptors, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. In each case, the fusion proteins may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric protein complex. In each case, purification of various heteromeric 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.

Table: "Type II:Type II Receptor Heterodimers"

Amino Acid SEQ ID NOs for Type II:Type II Receptor Heterodimers					
Heteromeric Fusion Protein Complex	Fc Pair Type	ECD Correspondence to Fc Pair	Receptor-Fc Fusion Protein	Amino Acid SEQ ID NO	
				With Leader	Mature
ActRIIA-Fc: ActRIIB-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			ActRIIB-Fc	153	154
		B	ActRIIA-Fc	151	152
			ActRIIB-Fc	100	102
	Hydrophobic	A	ActRIIA-Fc	409	410
			ActRIIB-Fc	453	454
		B	ActRIIA-Fc	451	452
			ActRIIB-Fc	401	402
ActRIIA-Fc: BMPRII-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			BMPRII-Fc	155	156
		B	ActRIIA-Fc	151	152

			BMPRII-Fc	121	123
	Hydrophobic	A	ActRIIA-Fc	409	410
			BMPRII-Fc	455	456
		B	ActRIIA-Fc	451	452
			BMPRII-Fc	411	412
ActRIIA-Fc: MISRII-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			MISRII-Fc	161	162
		B	ActRIIA-Fc	151	152
			MISRII-Fc	133	135
	Hydrophobic	A	ActRIIA-Fc	409	410
			MISRII-Fc	457	458
		B	ActRIIA-Fc	451	452
			MISRII-Fc	419	420
ActRIIA-Fc: $TGF\beta RII_{SHORT}$-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			$TGF\beta RII_{SHORT}$-Fc	157	158
		B	ActRIIA-Fc	151	152
			$TGF\beta RII_{SHORT}$-Fc	127	129
	Hydrophobic	A	ActRIIA-Fc	409	410
			$TGF\beta RII_{SHORT}$-Fc	459	460
		B	ActRIIA-Fc	451	452
			$TGF\beta RII_{SHORT}$-Fc	415	416
ActRIIA-Fc: $TGF\beta RII_{LONG}$-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			$TGF\beta RII_{LONG}$-Fc	159	160
		B	ActRIIA-Fc	151	152
			$TGF\beta RII_{LONG}$-Fc	130	132
	Hydrophobic	A	ActRIIA-Fc	409	410
			$TGF\beta RII_{LONG}$-Fc	461	462
		B	ActRIIA-Fc	451	452
			$TGF\beta RII_{LONG}$-Fc	417	418
ActRIIB-Fc: BMPRII-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			BMPRII-Fc	155	156
		B	ActRIIB-Fc	153	154
			BMPRII-Fc	121	123
	Hydrophobic	A	ActRIIB-Fc	401	402
			BMPRII-Fc	455	456
		B	ActRIIB-Fc	453	454
			BMPRII-Fc	411	412

ActRIIB-Fc: MISRII-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			MISRII-Fc	161	162
		B	ActRIIB-Fc	153	154
			MISRII-Fc	133	135
	Hydrophobic	A	ActRIIB-Fc	401	402
			MISRII-Fc	457	458
		B	ActRIIB-Fc	453	454
			MISRII-Fc	419	420
ActRIIB-Fc: $TGF\beta RII_{SHORT}$-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			$TGF\beta RII_{SHORT}$-Fc	157	158
		B	ActRIIB-Fc	153	154
			$TGF\beta RII_{SHORT}$-Fc	127	129
	Hydrophobic	A	ActRIIB-Fc	401	402
			$TGF\beta RII_{SHORT}$-Fc	459	460
		B	ActRIIB-Fc	453	454
			$TGF\beta RII_{SHORT}$-Fc	415	416
ActRIIB-Fc: $TGF\beta RII_{LONG}$-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			$TGF\beta RII_{LONG}$-Fc	159	160
		B	ActRIIB-Fc	153	154
			$TGF\beta RII_{LONG}$-Fc	130	132
	Hydrophobic	A	ActRIIB-Fc	401	402
			$TGF\beta RII_{LONG}$-Fc	461	462
		B	ActRIIB-Fc	453	454
			$TGF\beta RII_{LONG}$-Fc	417	418
BMPRII-Fc: MISRII-Fc	Electrostatic	A	BMPRII-Fc	121	123
			MISRII-Fc	161	162
		B	BMPRII-Fc	155	156
			MISRII-Fc	133	135
	Hydrophobic	A	BMPRII-Fc	411	412
			MISRII-Fc	457	458
		B	BMPRII-Fc	455	456
			MISRII-Fc	419	420
BMPRII-Fc: $TGF\beta RII_{SHORT}$-Fc	Electrostatic	A	BMPRII-Fc	121	123
			$TGF\beta RII_{SHORT}$-Fc	157	158
		B	BMPRII-Fc	155	156
			$TGF\beta RII_{SHORT}$-Fc	127	129
	Hydrophobic	A	BMPRII-Fc	411	412

			$TGF\beta RII_{SHORT}$-Fc	459	460
		B	BMPRII-Fc	455	456
			$TGF\beta RII_{SHORT}$-Fc	415	416
BMPRII-Fc: $TGF\beta RII_{LONG}$-Fc	Electrostatic	A	BMPRII-Fc	121	123
			$TGF\beta RII_{LONG}$-Fc	159	160
		B	BMPRII-Fc	155	156
			$TGF\beta RII_{LONG}$-Fc	130	132
	Hydrophobic	A	BMPRII-Fc	411	412
			$TGF\beta RII_{LONG}$-Fc	461	462
		B	BMPRII-Fc	455	456
			$TGF\beta RII_{LONG}$-Fc	417	418
MISRII-Fc: $TGF\beta RII_{SHORT}$-Fc	Electrostatic	A	MISRII-Fc	133	135
			$TGF\beta RII_{SHORT}$-Fc	157	158
		B	MISRII-Fc	161	162
			$TGF\beta RII_{SHORT}$-Fc	127	129
	Hydrophobic	A	MISRII-Fc	419	420
			$TGF\beta RII_{SHORT}$-Fc	459	460
		B	MISRII-Fc	457	458
			$TGF\beta RII_{SHORT}$-Fc	415	416
MISRII-Fc: $TGF\beta RII_{LONG}$-Fc	Electrostatic	A	MISRII-Fc	133	135
			$TGF\beta RII_{LONG}$-Fc	159	160
		B	MISRII-Fc	161	162
			$TGF\beta RII_{LONG}$-Fc	130	132
	Hydrophobic	A	MISRII-Fc	419	420
			$TGF\beta RII_{LONG}$-Fc	461	462
		B	MISRII-Fc	457	458
			$TGF\beta RII_{LONG}$-Fc	417	418
$TGF\beta RII_{SHORT}$-Fc: $TGF\beta RII_{LONG}$-Fc	Electrostatic	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			$TGF\beta RII_{LONG}$-Fc	159	160
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			$TGF\beta RII_{LONG}$-Fc	130	132
	Hydrophobic	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			$TGF\beta RII_{LONG}$-Fc	461	462
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			$TGF\beta RII_{LONG}$-Fc	417	418

Example 36. Generation of an ALK1-Fc:ALK2-Fc heterodimer

A soluble ALK1-Fc:ALK2-Fc heteromeric complex can be generated comprising the extracellular domains of human ALK1 and human ALK2, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ALK1-Fc and ALK2-Fc fusion proteins, respectively.

Formation of heteromeric ALK1-Fc:ALK2-Fc may be guided by approaches similar to those described in Example 1.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ALK1-Fc fusion protein and a nucleic acid sequence encoding it are provided above in Example 16 as SEQ ID NOs: 124-126.

The polypeptide sequence of the complementary ALK2-Fc fusion protein (SEQ ID NO: 173) employs the TPA leader and is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
301  KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPG      (SEQ ID NO: 173)
```

The leader and linker sequences are underlined. To promote formation of the ALK1-Fc:ALK2-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysines) 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: 173 may optionally be provided with a lysine added to the C-terminus.

The mature ALK2-Fc fusion protein sequence is as follows (SEQ ID NO: 174) and may optionally be provided with a lysine added to the C-terminus.

```
  1  MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
 51  FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
```

```
101  HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR KEMTKNQVSL
251  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF LYSKLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G    (SEQ ID NO: 174)
```

The ALK1-Fc and ALK2-Fc fusion proteins of SEQ ID NO: 126 and SEQ ID NO: 174, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ALK1-Fc:ALK2-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are both linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, an alternative polypeptide sequence of the ALK1-Fc fusion protein (SEQ ID NO: 171) 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
151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251  PQVYTLPPSR KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
301  PVLKSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  G          (SEQ ID NO: 171)
```

The leader and linker sequences are underlined. To promote formation of the ALK1-Fc:ALK2-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysines) 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: 171 may optionally be provided with a lysine added to the C-terminus.

The mature ALK1-Fc fusion protein sequence is as follows (SEQ ID NO: 172) and may optionally be provided with a lysine added to the C-terminus.

```
  1  DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
 51  LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
101  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
```

```
151  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
201  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRKEMT KNQVSLTCLV
251  KGFYPSDIAV EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ
301  GNVFSCSVMH EALHNHYTQK SLSLSPG          (SEQ ID NO: 172)
```

The polypeptide sequence of the ALK2-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 9 as SEQ ID NOs: 136-138.

The ALK1-Fc and ALK2-Fc fusion proteins of SEQ ID NO: 172 and SEQ ID NO: 138, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK2-Fc.

Hydrophobic Approach

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. The polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 16 as SEQ ID NOs: 413-414.

The complementary form of ALK2-Fc fusion polypeptide (SEQ ID NO: 465) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW ESNGQPENNY
301  KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPGK          (SEQ ID NO: 465)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ALK1-Fc fusion polypeptide of SEQ ID NOs: 413-414, 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 ALK2-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 465 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK2-Fc fusion protein sequence (SEQ ID NO: 466) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
 51  FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
101  HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR EEMTKNQVSL
251  WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK   (SEQ ID NO: 466)
```

The ALK1-Fc and ALK2-Fc proteins of SEQ ID NO: 414 and SEQ ID NO: 466, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK2-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, an alternative polypeptide sequence of the ALK1-Fc fusion protein (SEQ ID NO: 463) 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
151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251  PQVYTLPPCR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP
301  PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351  GK       (SEQ ID NO: 463)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ALK2-Fc fusion polypeptide of SEQ ID NOs 421-422, 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 ALK1-Fc fusion polypeptide as indicated by double underline above.

The amino acid sequence of SEQ ID NO: 463 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK1-Fc fusion protein sequence is as follows (SEQ ID NO: 464) and may optionally be provided with the lysine removed from the C-terminus.

```
  1  DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
 51  LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
101  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
151  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
201  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV
251  KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
301  GNVFSCSVMH EALHNHYTQK SLSLSPGK      (SEQ ID NO: 464)
```

The polypeptide sequence of the ALK2-Fc fusion protein is provided in Example 9 as SEQ ID NOs: 421-422.

The ALK1-Fc and ALK2-Fc proteins of SEQ ID NO: 464 and SEQ ID NO: 422, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK2-Fc.

Purification of various ALK1-Fc:ALK2-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 37. Generation of an ALK1-Fc:ALK3-Fc heterodimer

A soluble ALK1-Fc:ALK3-Fc heteromeric complex can be generated comprising the extracellular domains of human ALK1 and human ALK3, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ALK1-Fc and ALK3-Fc fusion proteins, respectively.

Formation of heteromeric ALK1-Fc:ALK3-Fc may be guided by approaches similar to those described in Example 36.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ALK1-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 16 as SEQ ID NOs: 124-126.

The polypeptide sequence of the complementary ALK3-Fc fusion protein (SEQ ID NO: 175) employs the TPA leader and is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51  LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
101  CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
151  SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR KEMTKNQVSL
301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF LYSKLTVDKS
351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G   (SEQ ID NO: 175)
```

The leader and linker sequences are underlined. To promote formation of the ALK1-Fc:ALK3-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysines) 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: 175 may optionally be provided with a lysine added to the C-terminus.

The mature ALK3-Fc fusion protein sequence is as follows (SEQ ID NO: 176) and may optionally be provided with a lysine added to the C-terminus.

```
  1  GAQNLDSMLH GTGMKSDSDQ KKSENGVTLA PEDTLPFLKC YCSGHCPDDA
 51  INNTCITNGH CFAIIEEDDQ GETTLASGCM KYEGSDFQCK DSPKAQLRRT
101  IECCRTNLCN QYLQPTLPPV VIGPFFDGSI RTGGGTHTCP PCPAPELLGG
151  PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201  KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251  KAKGQPREPQ VYTLPPSRKE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301  ENNYKTTPPV LKSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351  QKSLSLSPG            (SEQ ID NO: 176)
```

The ALK1-Fc and ALK3-Fc fusion proteins of SEQ ID NO: 126 and SEQ ID NO: 176, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ALK1-Fc:ALK3-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 171-172.

The polypeptide sequence of the ALK3-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 4 as SEQ ID NOs: 115-117.

The ALK1-Fc and ALK3-Fc fusion proteins of SEQ ID NO: 172 and SEQ ID NO: 117, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK3-Fc.

Hydrophobic Approach

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. The polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 16 as SEQ ID NOs 413-414.

The complementary form of ALK3-Fc fusion polypeptide (SEQ ID NO: 467) is as follows:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51 LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
101 CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
151 SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR EEMTKNQVSL
301 WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK  (SEQ ID NO: 467)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ALK1-Fc fusion polypeptide of SEQ ID NOs: 413-414, 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 ALK3-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 467 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK3-Fc fusion protein sequence (SEQ ID NO: 468) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  GAQNLDSMLH GTGMKSDSDQ KKSENGVTLA PEDTLPFLKC YCSGHCPDDA
 51  INNTCITNGH CFAIIEEDDQ GETTLASGCM KYEGSDFQCK DSPKAQLRRT
101  IECCRTNLCN QYLQPTLPPV VIGPFFDGSI RTGGGTHTCP PCPAPELLGG
151  PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201  KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251  KAKGQPREPQ VYTLPPCREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
301  ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351  QKSLSLSPGK              (SEQ ID NO: 468)
```

The ALK1-Fc and ALK3-Fc proteins of SEQ ID NO: 414 and SEQ ID NO: 468, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK3-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 463-464.

The polypeptide sequence of the ALK3-Fc fusion protein is provided in Example 4 as SEQ ID NOs: 407-408.

The ALK1-Fc and ALK3-Fc proteins of SEQ ID NO: 464 and SEQ ID NO: 408, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK3-Fc.

Purification of various ALK1-Fc:ALK3-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 38. Generation of an ALK1-Fc:ALK4-Fc heterodimer

A soluble ALK1-Fc:ALK4-Fc heteromeric complex can be generated comprising the extracellular domains of human ALK1 and human ALK4, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ALK1-Fc and ALK4-Fc fusion proteins, respectively.

Formation of heteromeric ALK1-Fc:ALK4-Fc may be guided by approaches similar to those described in Example 36.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ALK1-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 16 as SEQ ID NOs: 124-126.

The polypeptide sequence of the complementary ALK4-Fc fusion protein (SEQ ID NO: 177) employs the TPA leader and is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 51  GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101  YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
301  KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPG      (SEQ ID NO: 177)
```

The leader and linker sequences are underlined. To promote formation of the ALK1-Fc:ALK4-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysines) 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: 177 may optionally be provided with a lysine added to the C-terminus.

The mature ALK4-Fc fusion protein sequence is as follows (SEQ ID NO: 178) and may optionally be provided with a lysine added to the C-terminus.

```
  1  SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
 51  ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
```

```
101  PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR KEMTKNQVSL
251  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF LYSKLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G   (SEQ ID NO: 178)
```

The ALK1-Fc and ALK4-Fc fusion proteins of SEQ ID NO: 126 and SEQ ID NO: 178, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ALK1-Fc:ALK4-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 171-172.

The polypeptide sequence of the ALK4-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 1 as SEQ ID NOs: 104-106.

The ALK1-Fc and ALK4-Fc fusion proteins of SEQ ID NO: 172 and SEQ ID NO: 106, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK4-Fc.

IIydrophobic Approach

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. The polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 16 as SEQ ID NOs 413-414.

The complementary form of ALK4-Fc fusion polypeptide (SEQ ID NO: 469) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 51  GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101  YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW ESNGQPENNY
```

```
301  KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPGK     (SEQ ID NO: 469)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ALK1-Fc fusion polypeptide of SEQ ID NOs: 413-414, 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 ALK4-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 469 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK4-Fc fusion protein sequence (SEQ ID NO: 470) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
 51  ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101  PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR EEMTKNQVSL
251  WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 470)
```

The ALK1-Fc and ALK4-Fc proteins of SEQ ID NO: 414 and SEQ ID NO: 470, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK4-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 463-464.

The polypeptide sequence of the ALK4-Fc fusion protein is provided in Example 1 as SEQ ID NOs: 403-404.

The ALK1-Fc and ALK4-Fc proteins of SEQ ID NO: 464 and SEQ ID NO: 404, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK4-Fc.

Purification of various ALK1-Fc:ALK4-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 39. Generation of an ALK1-Fc:ALK5-Fc heterodimer

A soluble ALK1-Fc:ALK5-Fc heteromeric complex can be generated comprising the extracellular domains of human ALK1 and human ALK5, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ALK1-Fc and ALK5-Fc fusion proteins, respectively.

Formation of heteromeric ALK1-Fc:ALK5-Fc may be guided by approaches similar to those described in Example 36.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ALK1-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 16 as SEQ ID NOs: 124-126.

The polypeptide sequence of the complementary ALK5-Fc fusion protein (SEQ ID NO: 179) 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
251  EPQVYTLPPS RKEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT
301  PPVLKSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
351  PG   (SEQ ID NO: 179)
```

The leader and linker sequences are underlined. To promote formation of the ALK1-Fc:ALK5-Fc heterodimer rather than either of the possible homodimeric complexes, two

amino acid substitutions (replacing acidic amino acids with lysines) 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: 179 may optionally be provided with a lysine added to the C-terminus.

The mature ALK5-Fc fusion protein sequence is as follows (SEQ ID NO: 180) and may optionally be provided with a lysine added to the C-terminus.

```
  1  ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
 51  DLIPRDRPFV CAPSSKTGSV TTTYCCNQDH CNKIELPTTV KSSPGLGPVE
101  TGGGTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
151  EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
201  YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRKEM TKNQVSLTCL
251  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL KSDGSFFLYS KLTVDKSRWQ
301  QGNVFSCSVM HEALHNHYTQ KSLSLSPG   (SEQ ID NO: 180)
```

The ALK1-Fc and ALK5-Fc fusion proteins of SEQ ID NO: 126 and SEQ ID NO: 180, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ALK1-Fc:ALK5-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 171-172.

The polypeptide sequence of the ALK5-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 11 as SEQ ID NOs: 139-141.

The ALK1-Fc and ALK5-Fc fusion proteins of SEQ ID NO: 172 and SEQ ID NO: 141, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK5-Fc.

Hydrophobic Approach

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. The polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 16 as SEQ ID NOs 413-414.

The complementary form of ALK5-Fc fusion polypeptide (SEQ ID NO: 471) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
 51  GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
101  NQDHCNKIEL PTTVKSSPGL GPVETGGGTH TCPPCPAPEL LGGPSVFLFP
151  PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
201  QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
251  EPQVYTLPPC REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
301  PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
351  PGK       (SEQ ID NO: 471)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ALK1-Fc fusion polypeptide of SEQ ID NOs: 413-414, 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 ALK5-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 471 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK5-Fc fusion protein sequence (SEQ ID NO: 472) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
 51  DLIPRDRPFV CAPSSKTGSV TTTYCCNQDH CNKIELPTTV KSSPGLGPVE
101  TGGGTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
151  EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
201  YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPCREEM TKNQVSLWCL
251  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ
301  QGNVFSCSVM HEALHNHYTQ KSLSLSPGK      (SEQ ID NO: 472)
```

The ALK1-Fc and ALK5-Fc proteins of SEQ ID NO: 414 and SEQ ID NO: 472, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK5-Fc.

Reverse Hydrophobic Approach

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 described

immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 463-464.

The polypeptide sequence of the ALK5-Fc fusion protein is provided in Example 16 as SEQ ID NOs: 423-424.

The ALK1-Fc and ALK5-Fc proteins of SEQ ID NO: 464 and SEQ ID NO: 424, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK5-Fc.

Purification of various ALK1-Fc:ALK5-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 40. Generation of an ALK1-Fc:ALK6-Fc heterodimer

A soluble ALK1-Fc:ALK6-Fc heteromeric complex can be generated comprising the extracellular domains of human ALK1 and human ALK6, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ALK1-Fc and ALK6-Fc fusion proteins, respectively.

Formation of heteromeric ALK1-Fc:ALK6-Fc may be guided by approaches similar to those described in Example 36.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ALK1-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 16 as SEQ ID NOs: 124-126.

The polypeptide sequence of the complementary ALK6-Fc fusion protein (SEQ ID NO: 181) employs the TPA leader and is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51  EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLEGSDF QCRDTPIPHQ
101  RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
```

151 PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201 VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251 IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW
301 ESNGQPENNY KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351 LHNHYTQKSL SLSPG (SEQ ID NO: 181)

The leader and linker sequences are underlined. To promote formation of the ALK1-Fc:ALK6-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysines) 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: 181 may optionally be provided with a lysine added to the C-terminus.

The mature ALK6-Fc fusion protein sequence is as follows (SEQ ID NO: 182) and may optionally be provided with a lysine added to the C-terminus.

1 KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
51 LPVVTSGCLG LEGSDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101 KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251 KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF
301 LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
(SEQ ID NO: 182)

The ALK1-Fc and ALK6-Fc fusion proteins of SEQ ID NO: 126 and SEQ ID NO: 182, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ALK1-Fc:ALK6-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 171-172.

The polypeptide sequence of the ALK6-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 13 as SEQ ID NOs: 142-144.

The ALK1-Fc and ALK6-Fc fusion proteins of SEQ ID NO: 172 and SEQ ID NO: 144, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK6-Fc.

Hydrophobic Approach

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. The polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 16 as SEQ ID NOs 413-414.

The complementary form of ALK6-Fc fusion polypeptide (SEQ ID NO: 473) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51  EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLEGSDF QCRDTPIPHQ
101  RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151  PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201  VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251  IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW
301  ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351  LHNHYTQKSL SLSPGK        (SEQ ID NO: 473)
```

The leader and linker sequences are underlined. To guide heterodimer formation with the ALK1-Fc fusion polypeptide of SEQ ID NOs: 413-414, 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 ALK6-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 473 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK6-Fc fusion protein sequence (SEQ ID NO: 474) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1     KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC
FTMIEEDDSG
 51     LPVVTSGCLG LEGSDFQCRD TPIPHQRRSI ECCTERNECN
KDLHPTLPPL
101     KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP
KPKDTLMISR
```

151 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV

201 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR

251 EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF

301 LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK

(SEQ ID NO: 474)

The ALK1-Fc and ALK6-Fc proteins of SEQ ID NO: 414 and SEQ ID NO: 474, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK6-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 463-464.

The polypeptide sequence of the ALK6-Fc fusion protein is provided in Example 13 as SEQ ID NOs: 425-426.

The ALK1-Fc and ALK6-Fc proteins of SEQ ID NO: 464 and SEQ ID NO: 426, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK6-Fc.

Purification of various ALK1-Fc:ALK6-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 41. Generation of an ALK1-Fc:ALK7-Fc heterodimer

A soluble ALK1-Fc:ALK7-Fc heteromeric complex can be generated comprising the extracellular domains of human ALK1 and human ALK7, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ALK1-Fc and ALK7-Fc fusion proteins, respectively.

Formation of heteromeric ALK1-Fc:ALK7-Fc may be guided by approaches similar to those described in Example 36.

Electrostatic Approach

In a first approach, the polypeptide sequence of the ALK1-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 16 as SEQ ID NOs: 124-126.

The polypeptide sequence of the complementary ALK7-Fc fusion protein (SEQ ID NO: 183) employs the TPA leader and is as follows:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51 MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101 TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251 KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF
301 LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
    (SEQ ID NO: 183)
```

The leader and linker sequences are underlined. To promote formation of the ALK1-Fc:ALK7-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysines) 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: 183 may optionally be provided with a lysine added to the C-terminus.

The mature ALK7-Fc fusion protein sequence is expected to be as follows (SEQ ID NO: 184) and may optionally be provided with a lysine added to the C-terminus.

```
  1 GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51 CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
```

201 APIEKTISKA KGQPREPQVY TLPPSRKEMT KNQVSLTCLV KGFYPSDIAV
251 EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
301 EALHNHYTQK SLSLSPG (SEQ ID NO: 184)

The ALK1-Fc and ALK7-Fc fusion proteins of SEQ ID NO: 126 and SEQ ID NO: 184, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ALK1-Fc:ALK7-Fc.

Reverse Electrostatic Approach

In a reverse approach, receptor extracellular domains are linked to the opposite member of the Fc interaction pair compared to the first approach described above. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 171-172.

The polypeptide sequence of the ALK7-Fc fusion protein and a nucleic acid sequence encoding it are provided in Example 7 as SEQ ID NOs: 112-114.

The ALK1-Fc and ALK7-Fc fusion proteins of SEQ ID NO: 172 and SEQ ID NO: 114, respectively, may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK7-Fc.

IIydrophobic Approach

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. The polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 16 as SEQ ID NOs 413-414.

The complementary form of ALK7-Fc fusion polypeptide (SEQ ID NO: 475) is as follows:

1 MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
51 MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101 TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR
251 EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301 LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
(SEQ ID NO: 475)

The leader and linker sequences are underlined. To guide heterodimer formation with the ALK1-Fc fusion polypeptide of SEQ ID NOs: 413-414, 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 ALK7-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 475 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 476) is expected to be as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51  CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
201  APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPSDIAV
251  EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
301  EALHNHYTQK SLSLSPGK        (SEQ ID NO: 476)
```

The ALK1-Fc and ALK7-Fc proteins of SEQ ID NO: 414 and SEQ ID NO: 476, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK7-Fc.

Reverse Hydrophobic Approach

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 described immediately above. However, receptor extracellular domains in this approach are linked to the opposite member of the Fc interaction pair compared to the above approach. In this example, the alternative polypeptide sequence of the ALK1-Fc fusion protein is provided in Example 36 as SEQ ID NOs: 463-464.

The polypeptide sequence of the ALK7-Fc fusion protein is provided in Example 7 as SEQ ID NOs: 405-406.

The ALK1-Fc and ALK7-Fc proteins of SEQ ID NO: 464 and SEQ ID NO: 406, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a variant heteromeric complex comprising ALK1-Fc:ALK7-Fc.

Purification of various ALK1-Fc:ALK7-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

Example 42. Generation of additional heterodimers comprising two type I receptors

Formation of additional heterdimeric protein complexes comprising extracellular domains of two type I receptors may be guided by approaches similar to those described in Example 36. Amino acid SEQ ID NOs for these variant heterodimers are disclosed in the following table, which for completeness also includes the heterodimers already discussed that comprise two type I receptors (Examples 36-41). As disclosed in Examples 36-41, the C-terminal lysine of each receptor-Fc fusion protein may optionally be included or omitted.

In each case, a soluble heteromeric complex can be generated comprising the extracellular domains of two different type I receptors, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. In each case, the fusion proteins may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric protein complex. In each case, purification of various heteromeric protein 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.

Table: "Type I:Type I Receptor Heterodimers"

Amino Acid SEQ ID NOs for Type I:Type I Receptor Heterodimers					
Heteromeric Fusion Protein Complex	Fc Pair Type	ECD Correspondence to Fc Pair	Receptor-Fc Fusion Protein	Amino Acid SEQ ID NO	
				With Leader	Mature
ALK1-Fc: ALK2-Fc	Electrostatic	A	ALK1-Fc	124	126
			ALK2-Fc	173	174
		B	ALK1-Fc	171	172
			ALK2-Fc	136	138

	Hydrophobic	A	ALK1-Fc	413	414
			ALK2-Fc	465	466
		B	ALK1-Fc	463	464
			ALK2-Fc	421	422
ALK1-Fc: ALK3-Fc	Electrostatic	A	ALK1-Fc	124	126
			ALK3-Fc	175	176
		B	ALK1-Fc	171	172
			ALK3-Fc	115	117
	Hydrophobic	A	ALK1-Fc	413	414
			ALK3-Fc	467	468
		B	ALK1-Fc	463	464
			ALK3-Fc	407	408
ALK1-Fc: ALK4-Fc	Electrostatic	A	ALK1-Fc	124	126
			ALK4-Fc	177	178
		B	ALK1-Fc	171	172
			ALK4-Fc	104	106
	Hydrophobic	A	ALK1-Fc	413	414
			ALK4-Fc	469	470
		B	ALK1-Fc	463	464
			ALK4-Fc	403	404
ALK1-Fc: ALK5-Fc	Electrostatic	A	ALK1-Fc	124	126
			ALK5-Fc	179	180
		B	ALK1-Fc	171	172
			ALK5-Fc	139	141
	Hydrophobic	A	ALK1-Fc	413	414
			ALK5-Fc	471	472
		B	ALK1-Fc	463	464
			ALK5-Fc	423	424
ALK1-Fc: ALK6-Fc	Electrostatic	A	ALK1-Fc	124	126
			ALK6-Fc	181	182
		B	ALK1-Fc	171	172
			ALK6-Fc	142	144
	Hydrophobic	A	ALK1-Fc	413	414
			ALK6-Fc	473	474
		B	ALK1-Fc	463	464
			ALK6-Fc	425	426
ALK1-Fc:	Electrostatic	A	ALK1-Fc	124	126

ALK7-Fc			ALK7-Fc	183	184
		B	ALK1-Fc	171	172
			ALK7-Fc	112	114
	Hydrophobic	A	ALK1-Fc	413	414
			ALK7-Fc	475	476
		B	ALK1-Fc	463	464
			ALK7-Fc	405	406
ALK2-Fc: ALK3-Fc	Electrostatic	A	ALK2-Fc	136	138
			ALK3-Fc	175	176
		B	ALK2-Fc	173	174
			ALK3-Fc	115	117
	Hydrophobic	A	ALK2-Fc	421	422
			ALK3-Fc	467	468
		B	ALK2-Fc	465	466
			ALK3-Fc	407	408
ALK2-Fc: ALK4-Fc	Electrostatic	A	ALK2-Fc	136	138
			ALK4-Fc	177	178
		B	ALK2-Fc	173	174
			ALK4-Fc	104	106
	Hydrophobic	A	ALK2-Fc	421	422
			ALK4-Fc	469	470
		B	ALK2-Fc	465	466
			ALK4-Fc	403	404
ALK2-Fc: ALK5-Fc	Electrostatic	A	ALK2-Fc	136	138
			ALK5-Fc	179	180
		B	ALK2-Fc	173	174
			ALK5-Fc	139	141
	Hydrophobic	A	ALK2-Fc	421	422
			ALK5-Fc	471	472
		B	ALK2-Fc	465	466
			ALK5-Fc	423	424
ALK2-Fc: ALK6-Fc	Electrostatic	A	ALK2-Fc	136	138
			ALK6-Fc	181	182
		B	ALK2-Fc	173	174
			ALK6-Fc	142	144
	Hydrophobic	A	ALK2-Fc	421	422
			ALK6-Fc	473	474

		B	ALK2-Fc	465	466
			ALK6-Fc	425	426
ALK2-Fc: ALK7-Fc	Electrostatic	A	ALK2-Fc	136	138
			ALK7-Fc	183	184
		B	ALK2-Fc	173	174
			ALK7-Fc	112	114
	Hydrophobic	A	ALK2-Fc	421	422
			ALK7-Fc	475	476
		B	ALK2-Fc	465	466
			ALK7-Fc	405	406
ALK3-Fc: ALK4-Fc	Electrostatic	A	ALK3-Fc	115	117
			ALK4-Fc	177	178
		B	ALK3-Fc	175	176
			ALK4-Fc	104	106
	Hydrophobic	A	ALK3-Fc	407	408
			ALK4-Fc	469	470
		B	ALK3-Fc	467	468
			ALK4-Fc	403	404
ALK3-Fc: ALK5-Fc	Electrostatic	A	ALK3-Fc	115	117
			ALK5-Fc	179	180
		B	ALK3-Fc	175	176
			ALK5-Fc	139	141
	Hydrophobic	A	ALK3-Fc	407	408
			ALK5-Fc	471	472
		B	ALK3-Fc	467	468
			ALK5-Fc	423	424
ALK3-Fc: ALK6-Fc	Electrostatic	A	ALK3-Fc	115	117
			ALK6-Fc	181	182
		B	ALK3-Fc	175	176
			ALK6-Fc	142	144
	Hydrophobic	A	ALK3-Fc	407	408
			ALK6-Fc	473	474
		B	ALK3-Fc	467	468
			ALK6-Fc	425	426
ALK3-Fc: ALK7-Fc	Electrostatic	A	ALK3-Fc	115	117
			ALK7-Fc	183	184
		B	ALK3-Fc	175	176

			ALK7-Fc	112	114
	Hydrophobic	A	ALK3-Fc	407	408
			ALK7-Fc	475	476
		B	ALK3-Fc	467	468
			ALK7-Fc	405	406
ALK4-Fc: ALK5-Fc	Electrostatic	A	ALK4-Fc	104	106
			ALK5-Fc	179	180
		B	ALK4-Fc	177	178
			ALK5-Fc	139	141
	Hydrophobic	A	ALK4-Fc	403	404
			ALK5-Fc	471	472
		B	ALK4-Fc	469	470
			ALK5-Fc	423	424
ALK4-Fc: ALK6-Fc	Electrostatic	A	ALK4-Fc	104	106
			ALK6-Fc	181	182
		B	ALK4-Fc	177	178
			ALK6-Fc	142	144
	Hydrophobic	A	ALK4-Fc	403	404
			ALK6-Fc	473	474
		B	ALK4-Fc	469	470
			ALK6-Fc	425	426
ALK4-Fc: ALK7-Fc	Electrostatic	A	ALK4-Fc	104	106
			ALK7-Fc	183	184
		B	ALK4-Fc	177	178
			ALK7-Fc	112	114
	Hydrophobic	A	ALK4-Fc	403	404
			ALK7-Fc	475	476
		B	ALK4-Fc	469	470
			ALK7-Fc	405	406
ALK5-Fc: ALK6-Fc	Electrostatic	A	ALK5-Fc	139	141
			ALK6-Fc	181	182
		B	ALK5-Fc	179	180
			ALK6-Fc	142	144
	Hydrophobic	A	ALK5-Fc	423	424
			ALK6-Fc	473	474
		B	ALK5-Fc	471	472
			ALK6-Fc	425	426

ALK5-Fc: ALK7-Fc	Electrostatic	A	ALK5-Fc	139	141
			ALK7-Fc	183	184
		B	ALK5-Fc	179	180
			ALK7-Fc	112	114
	Hydrophobic	A	ALK5-Fc	423	424
			ALK7-Fc	475	476
		B	ALK5-Fc	471	472
			ALK7-Fc	405	406
ALK6-Fc: ALK7-Fc	Electrostatic	A	ALK6-Fc	142	144
			ALK7-Fc	183	184
		B	ALK6-Fc	181	182
			ALK7-Fc	112	114
	Hydrophobic	A	ALK6-Fc	425	426
			ALK7-Fc	475	476
		B	ALK6-Fc	473	474
			ALK7-Fc	405	406

Example 43. Generation of additional heterodimers comprising a type I receptor and a type II receptor

Formation of additional heterdimeric protein complexes comprising extracellular domains of a type I receptor and a type II receptor may be guided by approaches similar to those described in Example 1. Amino acid SEQ ID NOs for these variant heterodimers are disclosed in the following table, which for completeness also includes the heterodimers already discussed that comprise a type I receptor and a type II receptor (Examples 1-29). As disclosed in Examples 1-29, the C-terminal lysine of each receptor-Fc fusion protein may optionally be included or omitted.

In each case, a soluble heteromeric complex can be generated comprising the extracellular domains of a type I receptor and a type II receptor, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. In each case, the fusion proteins may be co-expressed and purified from a CHO cell line to give rise to a variant heteromeric protein complex. In each case, purification of various heteromeric protein 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.

Table: "Type I:Type II Receptor Heterodimers"

Amino Acid SEQ ID NOs for Type I:Type II Receptor Heterodimers					
Heteromeric Fusion Protein Complex	Fc Pair Type	ECD Correspondence to Fc Pair	Receptor-Fc Fusion Protein	Amino Acid SEQ ID NO	
				With Leader	Mature
ActRIIA-Fc: ALK1-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			ALK1-Fc	124	126
		B	ActRIIA-Fc	151	152
			ALK1-Fc	171	172
	Hydrophobic	A	ActRIIA-Fc	409	410
			ALK1-Fc	413	414
		B	ActRIIA-Fc	451	452
			ALK1-Fc	463	464
ActRIIA-Fc: ALK2-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			ALK2-Fc	136	138
		B	ActRIIA-Fc	151	152
			ALK2-Fc	173	174
	Hydrophobic	A	ActRIIA-Fc	409	410
			ALK2-Fc	421	422
		B	ActRIIA-Fc	451	452
			ALK2-Fc	465	466
ActRIIA-Fc: ALK3-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			ALK3-Fc	115	117
		B	ActRIIA-Fc	151	152
			ALK3-Fc	175	176
	Hydrophobic	A	ActRIIA-Fc	409	410
			ALK3-Fc	407	408
		B	ActRIIA-Fc	451	452
			ALK3-Fc	467	468
ActRIIA-Fc: ALK4-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			ALK4-Fc	104	106
		B	ActRIIA-Fc	151	152
			ALK4-Fc	177	178
	Hydrophobic	A	ActRIIA-Fc	409	410

			ALK4-Fc	403	404
		B	ActRIIA-Fc	451	452
			ALK4-Fc	469	470
ActRIIA-Fc: ALK5-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			ALK5-Fc	139	141
		B	ActRIIA-Fc	151	152
			ALK5-Fc	179	180
	Hydrophobic	A	ActRIIA-Fc	409	410
			ALK5-Fc	423	424
		B	ActRIIA-Fc	451	452
			ALK5-Fc	471	472
ActRIIA-Fc: ALK6-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			ALK6-Fc	142	144
		B	ActRIIA-Fc	151	152
			ALK6-Fc	181	182
	Hydrophobic	A	ActRIIA-Fc	409	410
			ALK6-Fc	425	426
		B	ActRIIA-Fc	451	452
			ALK6-Fc	473	474
ActRIIA-Fc: ALK7-Fc	Electrostatic	A	ActRIIA-Fc	118	120
			ALK7-Fc	112	114
		B	ActRIIA-Fc	151	152
			ALK7-Fc	183	184
	Hydrophobic	A	ActRIIA-Fc	409	410
			ALK7-Fc	405	406
		B	ActRIIA-Fc	451	452
			ALK7-Fc	475	476
ActRIIB-Fc: ALK1-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			ALK1-Fc	124	126
		B	ActRIIB-Fc	153	154
			ALK1-Fc	171	172
	Hydrophobic	A	ActRIIB-Fc	401	402
			ALK1-Fc	413	414
		B	ActRIIB-Fc	453	454
			ALK1-Fc	463	464
ActRIIB-Fc: ALK2-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			ALK2-Fc	136	138

		B	ActRIIB-Fc	153	154
			ALK2-Fc	173	174
	Hydrophobic	A	ActRIIB-Fc	401	402
			ALK2-Fc	421	422
		B	ActRIIB-Fc	453	454
			ALK2-Fc	465	466
ActRIIB-Fc: ALK3-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			ALK3-Fc	115	117
		B	ActRIIB-Fc	153	154
			ALK3-Fc	175	176
	Hydrophobic	A	ActRIIB-Fc	401	402
			ALK3-Fc	407	408
		B	ActRIIB-Fc	453	454
			ALK3-Fc	467	468
ActRIIB-Fc: ALK4-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			ALK4-Fc	104	106
		B	ActRIIB-Fc	153	154
			ALK4-Fc	177	178
	Hydrophobic	A	ActRIIB-Fc	401	402
			ALK4-Fc	403	404
		B	ActRIIB-Fc	453	454
			ALK4-Fc	469	470
ActRIIB-Fc: ALK5-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			ALK5-Fc	139	141
		B	ActRIIB-Fc	153	154
			ALK5-Fc	179	180
	Hydrophobic	A	ActRIIB-Fc	401	402
			ALK5-Fc	423	424
		B	ActRIIB-Fc	453	454
			ALK5-Fc	471	472
ActRIIB-Fc: ALK6-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			ALK6-Fc	142	144
		B	ActRIIB-Fc	153	154
			ALK6-Fc	181	182
	Hydrophobic	A	ActRIIB-Fc	401	402
			ALK6-Fc	425	426
		B	ActRIIB-Fc	453	454

			ALK6-Fc	473	474
ActRIIB-Fc: ALK7-Fc	Electrostatic	A	ActRIIB-Fc	100	102
			ALK7-Fc	112	114
		B	ActRIIB-Fc	153	154
			ALK7-Fc	183	184
	Hydrophobic	A	ActRIIB-Fc	401	402
			ALK7-Fc	405	406
		B	ActRIIB-Fc	453	454
			ALK7-Fc	475	476
BMPRII-Fc: ALK1-Fc	Electrostatic	A	BMPRII-Fc	121	123
			ALK1-Fc	124	126
		B	BMPRII-Fc	155	156
			ALK1-Fc	171	172
	Hydrophobic	A	BMPRII-Fc	411	412
			ALK1-Fc	413	414
		B	BMPRII-Fc	455	456
			ALK1-Fc	463	464
BMPRII-Fc: ALK2-Fc	Electrostatic	A	BMPRII-Fc	121	123
			ALK2-Fc	136	138
		B	BMPRII-Fc	155	156
			ALK2-Fc	173	174
	Hydrophobic	A	BMPRII-Fc	411	412
			ALK2-Fc	421	422
		B	BMPRII-Fc	455	456
			ALK2-Fc	465	466
BMPRII-Fc: ALK3-Fc	Electrostatic	A	BMPRII-Fc	121	123
			ALK3-Fc	115	117
		B	BMPRII-Fc	155	156
			ALK3-Fc	175	176
	Hydrophobic	A	BMPRII-Fc	411	412
			ALK3-Fc	407	408
		B	BMPRII-Fc	455	456
			ALK3-Fc	467	468
BMPRII-Fc: ALK4-Fc	Electrostatic	A	BMPRII-Fc	121	123
			ALK4-Fc	104	106
		B	BMPRII-Fc	155	156
			ALK4-Fc	177	178

	Hydrophobic	A	BMPRII-Fc	411	412
			ALK4-Fc	403	404
		B	BMPRII-Fc	455	456
			ALK4-Fc	469	470
BMPRII-Fc: ALK5-Fc	Electrostatic	A	BMPRII-Fc	121	123
			ALK5-Fc	139	141
		B	BMPRII-Fc	155	156
			ALK5-Fc	179	180
	Hydrophobic	A	BMPRII-Fc	411	412
			ALK5-Fc	423	424
		B	BMPRII-Fc	455	456
			ALK5-Fc	471	472
BMPRII-Fc: ALK6-Fc	Electrostatic	A	BMPRII-Fc	121	123
			ALK6-Fc	142	144
		B	BMPRII-Fc	155	156
			ALK6-Fc	181	182
	Hydrophobic	A	BMPRII-Fc	411	412
			ALK6-Fc	425	426
		B	BMPRII-Fc	455	456
			ALK6-Fc	473	474
BMPRII-Fc: ALK7-Fc	Electrostatic	A	BMPRII-Fc	121	123
			ALK7-Fc	112	114
		B	BMPRII-Fc	155	156
			ALK7-Fc	183	184
	Hydrophobic	A	BMPRII-Fc	411	412
			ALK7-Fc	405	406
		B	BMPRII-Fc	455	456
			ALK7-Fc	475	476
MISRII-Fc: ALK1-Fc	Electrostatic	A	MISRII-Fc	133	135
			ALK1-Fc	124	126
		B	MISRII-Fc	161	162
			ALK1-Fc	171	172
	Hydrophobic	A	MISRII-Fc	419	420
			ALK1-Fc	413	414
		B	MISRII-Fc	457	458
			ALK1-Fc	463	464
MISRII-Fc:	Electrostatic	A	MISRII-Fc	133	135

ALK2-Fc			ALK2-Fc	136	138
		B	MISRII-Fc	161	162
			ALK2-Fc	173	174
	Hydrophobic	A	MISRII-Fc	419	420
			ALK2-Fc	421	422
		B	MISRII-Fc	457	458
			ALK2-Fc	465	466
MISRII-Fc: ALK3-Fc	Electrostatic	A	MISRII-Fc	133	135
			ALK3-Fc	115	117
		B	MISRII-Fc	161	162
			ALK3-Fc	175	176
	Hydrophobic	A	MISRII-Fc	419	420
			ALK3-Fc	407	408
		B	MISRII-Fc	457	458
			ALK3-Fc	467	468
MISRII-Fc: ALK4-Fc	Electrostatic	A	MISRII-Fc	133	135
			ALK4-Fc	104	106
		B	MISRII-Fc	161	162
			ALK4-Fc	177	178
	Hydrophobic	A	MISRII-Fc	419	420
			ALK4-Fc	403	404
		B	MISRII-Fc	457	458
			ALK4-Fc	469	470
MISRII-Fc: ALK5-Fc	Electrostatic	A	MISRII-Fc	133	135
			ALK5-Fc	139	141
		B	MISRII-Fc	161	162
			ALK5-Fc	179	180
	Hydrophobic	A	MISRII-Fc	419	420
			ALK5-Fc	423	424
		B	MISRII-Fc	457	458
			ALK5-Fc	471	472
MISRII-Fc: ALK6-Fc	Electrostatic	A	MISRII-Fc	133	135
			ALK6-Fc	142	144
		B	MISRII-Fc	161	162
			ALK6-Fc	181	182
	Hydrophobic	A	MISRII-Fc	419	420
			ALK6-Fc	425	426

		B	MISRII-Fc	457	458
			ALK6-Fc	473	474
MISRII-Fc: ALK7-Fc	Electrostatic	A	MISRII-Fc	133	135
			ALK7-Fc	112	114
		B	MISRII-Fc	161	162
			ALK7-Fc	183	184
	Hydrophobic	A	MISRII-Fc	419	420
			ALK7-Fc	405	406
		B	MISRII-Fc	457	458
			ALK7-Fc	475	476
$TGF\beta RII_{SHORT}$-Fc: ALK1-Fc	Electrostatic	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK1-Fc	124	126
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK1-Fc	171	172
	Hydrophobic	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK1-Fc	413	414
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK1-Fc	463	464
$TGF\beta RII_{SHORT}$-Fc: ALK2-Fc	Electrostatic	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK2-Fc	136	138
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK2-Fc	173	174
	Hydrophobic	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK2-Fc	421	422
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK2-Fc	465	466
$TGF\beta RII_{SHORT}$-Fc: ALK3-Fc	Electrostatic	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK3-Fc	115	117
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK3-Fc	175	176
	Hydrophobic	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK3-Fc	407	408
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK3-Fc	467	468
$TGF\beta RII_{SHORT}$-Fc: ALK4-Fc	Electrostatic	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK4-Fc	104	106
		B	$TGF\beta RII_{SHORT}$-Fc	157	158

			ALK4-Fc	177	178
	Hydrophobic	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK4-Fc	403	404
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK4-Fc	469	470
$TGF\beta RII_{SHORT}$-Fc: ALK5-Fc	Electrostatic	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK5-Fc	139	141
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK5-Fc	179	180
	Hydrophobic	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK5-Fc	423	424
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK5-Fc	471	472
$TGF\beta RII_{SHORT}$-Fc: ALK6-Fc	Electrostatic	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK6-Fc	142	144
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK6-Fc	181	182
	Hydrophobic	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK6-Fc	425	426
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK6-Fc	473	474
$TGF\beta RII_{SHORT}$-Fc: ALK7-Fc	Electrostatic	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK7-Fc	112	114
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK7-Fc	183	184
	Hydrophobic	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK7-Fc	405	406
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK7-Fc	475	476
$TGF\beta RII_{LONG}$-Fc: ALK1-Fc	Electrostatic	A	$TGF\beta RII_{LONG}$-Fc	130	132
			ALK1-Fc	124	126
		B	$TGF\beta RII_{LONG}$-Fc	159	160
			ALK1-Fc	171	172
	Hydrophobic	A	$TGF\beta RII_{LONG}$-Fc	417	418
			ALK1-Fc	413	414
		B	$TGF\beta RII_{LONG}$-Fc	461	462
			ALK1-Fc	463	464

TGFβRII$_{LONG}$-Fc: ALK2-Fc	Electrostatic	A	TGFβRII$_{LONG}$-Fc	130	132
			ALK2-Fc	136	138
		B	TGFβRII$_{LONG}$-Fc	159	160
			ALK2-Fc	173	174
	Hydrophobic	A	TGFβRII$_{LONG}$-Fc	417	418
			ALK2-Fc	421	422
		B	TGFβRII$_{LONG}$-Fc	461	462
			ALK2-Fc	465	466
TGFβRII$_{LONG}$-Fc: ALK3-Fc	Electrostatic	A	TGFβRII$_{LONG}$-Fc	130	132
			ALK3-Fc	115	117
		B	TGFβRII$_{LONG}$-Fc	159	160
			ALK3-Fc	175	176
	Hydrophobic	A	TGFβRII$_{LONG}$-Fc	417	418
			ALK3-Fc	407	408
		B	TGFβRII$_{LONG}$-Fc	461	462
			ALK3-Fc	467	468
TGFβRII$_{LONG}$-Fc: ALK4-Fc	Electrostatic	A	TGFβRII$_{LONG}$-Fc	130	132
			ALK4-Fc	104	106
		B	TGFβRII$_{LONG}$-Fc	159	160
			ALK4-Fc	177	178
	Hydrophobic	A	TGFβRII$_{LONG}$-Fc	417	418
			ALK4-Fc	403	404
		B	TGFβRII$_{LONG}$-Fc	461	462
			ALK4-Fc	469	470
TGFβRII$_{LONG}$-Fc: ALK5-Fc	Electrostatic	A	TGFβRII$_{LONG}$-Fc	130	132
			ALK5-Fc	139	141
		B	TGFβRII$_{LONG}$-Fc	159	160
			ALK5-Fc	179	180
	Hydrophobic	A	TGFβRII$_{LONG}$-Fc	417	418
			ALK5-Fc	423	424
		B	TGFβRII$_{LONG}$-Fc	461	462
			ALK5-Fc	471	472
TGFβRII$_{LONG}$-Fc: ALK6-Fc	Electrostatic	A	TGFβRII$_{LONG}$-Fc	130	132
			ALK6-Fc	142	144
		B	TGFβRII$_{LONG}$-Fc	159	160
			ALK6-Fc	181	182
	Hydrophobic	A	TGFβRII$_{LONG}$-Fc	417	418

			ALK6-Fc	425	426
		B	$TGF\beta RII_{LONG}$-Fc	461	462
			ALK6-Fc	473	474
$TGF\beta RII_{LONG}$-Fc: ALK7-Fc	Electrostatic	A	$TGF\beta RII_{LONG}$-Fc	130	132
			ALK7-Fc	112	114
		B	$TGF\beta RII_{LONG}$-Fc	159	160
			ALK7-Fc	183	184
	Hydrophobic	A	$TGF\beta RII_{LONG}$-Fc	417	418
			ALK7-Fc	405	406
		B	$TGF\beta RII_{LONG}$-Fc	461	462
			ALK7-Fc	475	476

In their entirety, these examples demonstrate that type I and type II receptor polypeptides, when placed in the context of a heteromeric complex, form novel binding pockets that exhibit altered selectivity relative to either type of homomeric complex, allowing the formation of novel protein agents for possible use as therapeutic agents.

We claim:

1. A recombinant heteromultimer complex comprising a TGFβ superfamily type I receptor polypeptide, optionally an extracellular domain of a TGFβ superfamily type I receptor, and a TGFβ superfamily type II receptor polypeptide, optionally an extracellular domain of a TGFβ superfamily type II receptor, wherein the TGFβ superfamily type I receptor polypeptide is selected from: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7, and wherein the TGFβ superfamily type II receptor polypeptide is selected from: ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII.

2. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK1 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIA polypeptide.

3. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK2 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIA polypeptide.

4. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK3 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIA polypeptide.

5. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK4 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIA polypeptide.

6. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK5 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIA polypeptide.

7. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK6 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIA polypeptide.

8. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK7 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIA polypeptide.

9. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK1 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIB polypeptide.

10. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK2 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIB polypeptide.

11. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK3 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIB polypeptide.

12. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK4 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIB polypeptide.

13. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK5 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIB polypeptide.

14. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK6 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIB polypeptide.

15. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK7 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is an ActRIIB polypeptide.

16. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK1 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a TGFBRII polypeptide.

17. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK2 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a TGFBRII polypeptide.

18. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK3 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a TGFBRII polypeptide.

19. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK4 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a TGFBRII polypeptide.

20. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK5 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a TGFBRII polypeptide.

21. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK6 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a TGFBRII polypeptide.

22. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK7 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a TGFBRII polypeptide.

23. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK1 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a BMPRII polypeptide.

24. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK2 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a BMPRII polypeptide.

25. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK3 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a BMPRII polypeptide.

26. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK4 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a BMPRII polypeptide.

27. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK5 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a BMPRII polypeptide.

28. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK6 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a BMPRII polypeptide.

29. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK7 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a BMPRII polypeptide.

30. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK1 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a MISRII polypeptide.

31. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK2 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a MISRII polypeptide.

32. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK3 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a MISRII polypeptide.

33. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK4 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a MISRII polypeptide.

34. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK5 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a MISRII polypeptide.

35. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK6 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a MISRII polypeptide.

36. The heteromultimer complex of claim 1, wherein the TGFβ superfamily type I receptor polypeptide is an ALK7 polypeptide, and wherein the TGFβ superfamily type II receptor polypeptide is a MISRII polypeptide.

37. The heteromultimer complex of any one of claims 2, 9, 16, 23, or 30, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 15.

38. The heteromultimer complex of any one of claims 2, 9, 16, 23, or 30, wherein the ALK1 polypeptide comprises, consists, or consists essentially of the amino acid sequence of SEQ ID NO: 15.

39. The heteromultimer complex of any one of claims 3, 10, 17, 24, or 31, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 19.

40. The heteromultimer complex of any one of claims 3, 10, 17, 24, or 31, wherein the ALK2 polypeptide comprises, consists, or consists essentially of the amino acid sequence of SEQ ID NO: 19.

41. The heteromultimer complex of any one of claims 4, 11, 18, 25, or 32, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to amino acids 31-140 of SEQ ID NO: 22.

42. The heteromultimer complex of any one of claims 4, 11, 18, 25, or 32, wherein the ALK3 polypeptide comprises, consists, or consists essential of amino acids 31-140 of SEQ ID NO: 22.

43. The heteromultimer complex of any one of claims 4, 11, 18, 25, or 32, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 23.

44. The heteromultimer complex of any one of claims 4, 11, 18, 25, or 32, wherein the ALK3 polypeptide comprises, consists, or consists essentially of the amino acid sequence of SEQ ID NO: 23.

45. The heteromultimer complex of any one of claims 5, 12, 19, 26, or 33, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence selected from SEQ ID NO: 27, 84, 104, 106, 403, and 404.

46. The heteromultimer complex of any one of claims 5, 12, 19, 26, or 33, wherein the ALK4 polypeptide comprises, consists, or consists essentially of the amino acid sequence selected from SEQ ID NO: 27, 84, 104, 106, 403, and 404

47. The heteromultimer complex of any one of claims 6, 13, 20, 27, or 34, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 31 or 88.

48. The heteromultimer complex of any one of claims 6, 13, 20, 27, or 34, wherein the ALK5 polypeptide comprises, consists, or consists essentially of the amino acid sequence of SEQ ID NO: 31 or 88.

49. The heteromultimer complex of any one of claims 7, 14, 21, 28, or 35, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 35 or 92.

50. The heteromultimer complex of any one of claims 7, 14, 21, 28, or 35, wherein the ALK6 polypeptide comprises, consists, or consists essentially of the amino acid sequence of SEQ ID NO: 35 or 92.

51. The heteromultimer complex of any one of claims 8, 15, 22, 29, or 36, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence selected from SEQ ID NO: 39, 112, 114, 302, 306, 310, 313, 405, and 406.

52. The heteromultimer complex of any one of claims 8, 15, 22, 29, or 36, wherein the ALK7 polypeptide comprises, consists, or consists essentially of the amino acid sequence selected from SEQ ID NO: 39, 112, 114, 302, 306, 310, 313, 405, and 406.

53. The heteromultimer complex of any one of claims 2-8, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to a sequence selected from SEQ ID NOs: 10 or 11.

54. The heteromultimer complex of any one of claims 2-8, wherein the ActRIIA polypeptide comprises, consists, or consists essentially of an amino acid sequence selected from SEQ ID NOs: 10 or 11.

55. The heteromultimer complex of any one of claims 9-15, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to a sequence selected from SEQ ID NOs: 2, 3, 5, 6, 100, 102, 401, and 402.

56. The heteromultimer complex of any one of claims 9-15, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to a sequence selected from SEQ ID NOs: 2, 3, 5, or 6, and 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).

57. The heteromultimer complex of any one of claims 9-15, wherein the ActRIIB polypeptide comprises, consists, or consists essentially of an amino acid sequence selected from SEQ ID NOs: 2, 3, 5, 6, 100, 102, 401, and 402.

58. The heteromultimer complex of any one of claims 9-15, wherein the ActRIIB polypeptide comprises, consists, or consists essentially of an amino acid sequence selected from SEQ ID NOs: 2, 3, 5, or 6, and 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).

59. The heteromultimer complex of any one of claims 9-15, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO:1.

60. The heteromultimer complex of any one of claims 9-15, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99%, or 100% identical to amino acids 29-109 of SEQ ID NO:1, and 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).

61. The heteromultimer complex of any one of claims 9-15, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO:1.

62. The heteromultimer complex of any one of claims 9-15, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO:1, and 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).

63. The heteromultimer complex of any one of claims 16-22, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% to the sequence of SEQ ID NO: 43, 68, 129, or 416.

64. The heteromultimer complex of any one of claims 16-22, wherein the TGFBRII polypeptide comprises, consists, or consists essentially of the amino acid sequence of SEQ ID NO: 43, 68, 129, or 416.

65. The heteromultimer complex of any one of claims 23-29, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 47, 72, 123, or 412.

66. The heteromultimer complex of any one of claims 23-29, wherein the BMPRII polypeptide comprises, consists, or consists essentially of the amino acid sequence of SEQ ID NO: 47, 72, 123, or 412.

67. The heteromultimer complex of any one of claims 30-36, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 51, 76, or 80.

68. The heteromultimer complex of any one of claims 30-36, wherein the MISRII polypeptide comprises, consists, or consists essentially of the amino acid sequence of SEQ ID NO: 51, 76, or 80.

69. The heteromultimer complex of any one of claims 1-68, wherein the complex is a heterodimer.

70. The heteromultimer complex of any one of claims 1-69, wherein the extracellular domain of a TGFβ superfamily type I receptor polypeptide is a fusion protein further comprising at least one heterologous portion, optionally a first or second member of an interaction pair.

71. The heteromultimer complex of any one of claims 1-70, wherein the extracellular domain of a TGFβ superfamily type II receptor polypeptide is a fusion protein further comprising at least one heterologous portion, optionally a first or second member of an interaction pair.

72. The heteromultimer complex of claim 70 or 71, wherein the heterologous portion comprises a constant region from an IgG heavy chain.

73. The heteromultimer complex of claim 72, wherein the constant region from an IgG heavy chain is an immunoglobulin Fc domain.

74. The heteromultimer complex of claim 72 or 73, wherein the heterologous portion comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% to a sequence selected from any one of SEQ ID NOs: 200-212.

75. The heteromultimer complex of claim 73, wherein the immunoglobulin Fc domain comprises, consists, or consists essentially of an amino acid sequence selected from any one of SEQ ID NOs: 200-212.

76. The heteromultimer complex of any one of claims 73-75, wherein the immunoglobulin Fc domain comprises one or more amino acid mutations that promote heterodimer formation.

77. The heteromultimer complex of any one of claims 73-76, wherein the immunoglobulin Fc domain comprises one or more amino acid mutations that inhibit homodimer formation.

78. The heteromultimer complex of any one of claims 1-77, wherein the extracellular domain of a TGFβ superfamily type I receptor 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.

79. The heteromultimer complex of any one of claims 1-78, wherein the extracellular domain of a TGFβ superfamily type II receptor 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.

80. The heteromultimer complex of claim 78, wherein the TGFβ superfamily type I receptor polypeptide is glycosylated and has a glycosylation pattern obtainable from expression of the type I receptor polypeptide in a CHO cell.

81. The heteromultimer complex of claim 79, wherein the TGFβ superfamily type II receptor polypeptide is glycosylated and has a glycosylation pattern obtainable from expression of the type I receptor polypeptide in a CHO cell.

82. The heteromultimer complex of any one of claims 1-81, wherein the heteromultimer complex had one or more of the following characteristics:

i) binds to a TGF-beta superfamily ligand with a K_D of at least 10^{-7} M; and

ii) inhibits a TGFβ superfamily type I and/or type II receptor-mediated signaling transduction a cell.

83. The heteromultimer complex of any one of claims 1-82, wherein the heteromultimer complex binds to one or more of: BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A,

activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty.

84. The heteromultimer complex of any one of claims 1-83, wherein the heteromultimer complex inhibits the activity of one or more TGF-beta superfamily ligand in a cell-based assay.

85. The heteromultimer complex of claim 84, wherein the TGF-beta superfamily ligand is selected from: BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty.

86. A pharmaceutical preparation comprising the heteromultimer complex of any one of claims 1-85 and a pharmaceutically acceptable carrier.

87. A method for treating a patient having a disorder associated with muscle loss or insufficient muscle growth, comprising administering to a patient in need thereof an effective amount of the heteromultimer complex of any one of claims 1-85.

88. The method of claim 87, wherein the patient has muscle atrophy.

89. The method of claim 87, wherein the patient has a muscular dystrophy.

90. The method of claim 89, wherein the muscular dystrophy is Duchenne muscular dystrophy.

91. The method of claim 90, wherein the patient is a juvenile and treatment begins within one to five years of the date of diagnosis with Duchenne muscular dystrophy.

92. The method of claim 89, wherein the muscular dystrophy is facioscapulohumeral muscular dystrophy.

93. The method of claim 87, wherein the patient has amyotrophic lateral sclerosis.

94. The method of claim 93, wherein the patient receives treatment after diagnosis with amyotrophic lateral sclerosis.

95. The method of claim 87, wherein the disorder is cachexia associated with cancer or cancer therapy.

96. A method for treating a patient having a disorder associated with neurodegeneration, comprising administering to the patient in need thereof an effective amount of the heteromultimer complex of any one of claims 1-85.

97. The method of claim 96, wherein the disorder is amyotrophic lateral sclerosis.

98. A method for decreasing body fat content or reducing the rate of increase in body fat content in a subject, comprising administering to a subject in need thereof an effective amount of the heteromultimer complex of any one of claims 1-85.

99. A method for treating a disorder associated with undesirable body weight gain in a subject, comprising administering to a subject in need thereof an effective amount of the heteromultimer complex of any one of claims 1-85.

100. The method of claim 99, wherein said disorder is selected from: obesity, non-insulin dependent diabetes mellitus (NIDDM), cardiovascular disease, hypertension, osteoarthritis, stroke, respiratory problems, and gall bladder disease.

101. A method for reducing cholesterol and/or triglycerides in a patient, comprising administering to a subject patient in need thereof an effective amount of the heteromultimer complex of any one of claims 1-85.

102. A method for treating sarcopenia, comprising administering to a patient in need thereof an effective amount of the heteromultimer complex of any one of claims 1-85.

103. A recombinant heteromultimer comprising an ALK1 polypeptide and an ActRIIA polypeptide.

104. The heteromultimer of claim 103, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9.

105. The heteromultimer of claim 103, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 9.

106. The heteromultimer of claim 103, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

107. The heteromultimer of any one of claims 103-106, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452.

108. The heteromultimer of claim 103-106, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-95 of SEQ ID NO: 14.

109. The heteromultimer of claim 103-106, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22-118 of SEQ ID NO: 14.

110. The heteromultimer of claim 103-106, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

111. The heteromultimer of claim 103-106, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464.

112. A recombinant heteromultimer comprising an ALK2 polypeptide and an ActRIIA polypeptide.

113. The heteromultimer of claim 112, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9.

114. The heteromultimer of claim 112, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 9.

115. The heteromultimer of claim 112, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

116. The heteromultimer of any one of claims 112-115, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452.

117. The heteromultimer of claim 112-115, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 35-99 of SEQ ID NO: 18.

118. The heteromultimer of claim 112-115, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-123 of SEQ ID NO: 18.

119. The heteromultimer of claim 112-115, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

120. The heteromultimer of claim 112-115, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466.

121. A recombinant heteromultimer comprising an ALK3 polypeptide and an ActRIIA polypeptide.

122. The heteromultimer of claim 121, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9.

123. The heteromultimer of claim 121, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 9.

124. The heteromultimer of claim 121, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

125. The heteromultimer of any one of claims 121-124, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452.

126. The heteromultimer of claim 121-124, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 61-130 of SEQ ID NO: 22.

127. The heteromultimer of claim 121-124, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-152 of SEQ ID NO: 22.

128. The heteromultimer of claim 121-124, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

129. The heteromultimer of claim 121-124, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468.

130. A recombinant heteromultimer comprising an ALK4 polypeptide and an ActRIIA polypeptide.

131. The heteromultimer of claim 130, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9.

132. The heteromultimer of claim 130, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 9.

133. The heteromultimer of claim 130, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

134. The heteromultimer of any one of claims 130-133, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452.

135. The heteromultimer of claim 130-133, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-101 of SEQ ID NOs: 26 or 83.

136. The heteromultimer of claim 130-133, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-126 of SEQ ID Nos: 26 or 83.

137. The heteromultimer of claim 130-133, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-34 (e.g., amino acid residues 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34) SEQ ID NO: 26 or 83, and

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.

138. The heteromultimer of claim 130-133, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470.

139. A recombinant heteromultimer comprising an ALK5 polypeptide and an ActRIIA polypeptide.

140. The heteromultimer of claim 139, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9.

141. The heteromultimer of claim 139, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 9.

142. The heteromultimer of claim 139, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

143. The heteromultimer of any one of claims 139-142, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452.

144. The heteromultimer of claim 139-142, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 36-106 of SEQ ID NOs: 30 or 87.

145. The heteromultimer of claim 139-142, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-126 of SEQ ID NOs: 30 or 87.

146. The heteromultimer of claim 139-142, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

147. The heteromultimer of claim 139-142, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472.

148. A recombinant heteromultimer comprising an ALK6 polypeptide and an ActRIIA polypeptide.

149. The heteromultimer of claim 148, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9.

150. The heteromultimer of claim 148, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 9.

151. The heteromultimer of claim 148, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

152. The heteromultimer of any one of claims 148-151, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452.

153. The heteromultimer of claim 148-151, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 32-102 of SEQ ID NO: 34.

154. The heteromultimer of claim 148-151, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 14-126 of SEQ ID NO: 34.

155. The heteromultimer of claim 148-151, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

156. The heteromultimer of claim 148-151, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474.

157. The heteromultimer of claim 148-151, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 62-132 of SEQ ID NO: 91.

158. The heteromultimer of claim 148-151, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 26-156 of SEQ ID NO: 91.

159. The heteromultimer of claim 148-151, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

160. A recombinant heteromultimer comprising an ALK7 polypeptide and an ActRIIA polypeptide.

161. The heteromultimer of claim 160, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9.

162. The heteromultimer of claim 160, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 9.

163. The heteromultimer of claim 160, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

164. The heteromultimer of any one of claims 160-163, wherein the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 9, 10, 11, 118, 120, 151, 152, 409, 410, 451, and 452.

165. The heteromultimer of claim 160-163, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NOs: 38, 305, or 309.

166. The heteromultimer of claim 160-163, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-133 of SEQ ID NOs: 38, 305, or 309.

167. The heteromultimer of claim 160-163, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 21-28 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, and 28) SEQ ID NO: 38, 305, or 309, and

b) ends at any one of amino acids 92-133 (e.g., amino acid residues 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, and 133) of SEQ ID NO: 38, 305, or 309.

168. The heteromultimer of claim 160-163, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

169. A recombinant heteromultimer comprising an ALK1 polypeptide and an ActRIIB polypeptide.

170. The heteromultimer of claim 169, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1.

171. The heteromultimer of claim 169, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1.

172. The heteromultimer of claim 169, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

173. The heteromultimer of any one of claim 172, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454.

174. The heteromultimer of claim 169-172, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-95 of SEQ ID NO: 14.

175. The heteromultimer of claim 169-172, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22-118 of SEQ ID NO: 14.

176. The heteromultimer of claim 169-172, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

177. The heteromultimer of claim 169-172, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464.

178. A recombinant heteromultimer comprising an ALK2 polypeptide and an ActRIIB polypeptide.

179. The heteromultimer of claim 178, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1.

180. The heteromultimer of claim 178, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1.

181. The heteromultimer of claim 178, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

182. The heteromultimer of any one of claim 178, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454.

183. The heteromultimer of claim 178-182, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 35-99 of SEQ ID NO: 18.

184. The heteromultimer of claim 178-182, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-123 of SEQ ID NO: 18.

185. The heteromultimer of claim 178-182, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

186. The heteromultimer of claim 178-182, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466.

187. A recombinant heteromultimer comprising an ALK3 polypeptide and an ActRIIB polypeptide.

188. The heteromultimer of claim 187, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1.

189. The heteromultimer of claim 187, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1.

190. The heteromultimer of claim 187, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

191. The heteromultimer of any one of claim 187, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454.

192. The heteromultimer of claim 187-190, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 61-130 of SEQ ID NO: 22.

193. The heteromultimer of claim 187-190, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-152 of SEQ ID NO: 22.

194. The heteromultimer of claim 187-190, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

195. The heteromultimer of claim 187-190, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468.

196. A recombinant heteromultimer comprising an ALK4 polypeptide and an ActRIIB polypeptide.

197. The heteromultimer of claim 196, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1.

198. The heteromultimer of claim 196, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1.

199. The heteromultimer of claim 196, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

200. The heteromultimer of any one of claim 196, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454.

201. The heteromultimer of claim 196-200, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-101 of SEQ ID NOs: 26 or 83.

202. The heteromultimer of claim 130-133, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-126 of SEQ ID Nos: 26 or 83.

203. The heteromultimer of claim 196-200, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-34 (e.g., amino acid residues 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34) SEQ ID NO: 26 or 83, and

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.

204. The heteromultimer of claim 196-200, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470.

205. A recombinant heteromultimer comprising an ALK5 polypeptide and an ActRIIB polypeptide.

206. The heteromultimer of claim 205, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1.

207. The heteromultimer of claim 205, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1.

208. The heteromultimer of claim 205, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

209. The heteromultimer of any one of claim 205, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454.

210. The heteromultimer of claim 205-209, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 36-106 of SEQ ID NOs: 30 or 87.

211. The heteromultimer of claim 205-209, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-126 of SEQ ID NOs: 30 or 87.

212. The heteromultimer of claim 205-209, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

213. The heteromultimer of claim 205-209, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472.

214. A recombinant heteromultimer comprising an ALK6 polypeptide and an ActRIIB polypeptide.

215. The heteromultimer of claim 214, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1.

216. The heteromultimer of claim 214, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1.

217. The heteromultimer of claim 214, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

218. The heteromultimer of any one of claim 214, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454.

219. The heteromultimer of claim 214-218, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 32-102 of SEQ ID NO: 34.

220. The heteromultimer of claim 214-218, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 14-126 of SEQ ID NO: 34.

221. The heteromultimer of claim 214-218, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

222. The heteromultimer of claim 214-218, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474.

223. The heteromultimer of claim 214-218, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 62-132 of SEQ ID NO: 91.

224. The heteromultimer of claim 214-218, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 26-156 of SEQ ID NO: 91.

225. The heteromultimer of claim 214-218, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

226. A recombinant heteromultimer comprising an ALK7 polypeptide and an ActRIIB polypeptide.

227. The heteromultimer of claim 226, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1.

228. The heteromultimer of claim 226, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1.

229. The heteromultimer of claim 226, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

230. The heteromultimer of any one of claim 226, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 1, 2, 3, 4, 5, 6, 100, 102, 153, 154, 401, 402, 453, and 454.

231. The heteromultimer of claim 226-230, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NOs: 38, 305, or 309.

232. The heteromultimer of claim 226-230, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-133 of SEQ ID NOs: 38, 305, or 309.

233. The heteromultimer of claim 226-230, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 21-28 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, and 28) SEQ ID NO: 38, 305, or 309, and

b) ends at any one of amino acids 92-133 (e.g., amino acid residues 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, and 133) of SEQ ID NO: 38, 305, or 309.

234. The heteromultimer of claim 226-230, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

235. A recombinant heteromultimer comprising an ALK1 polypeptide and a BMPRII polypeptide.

236. The heteromultimer of claim 235, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-123 of SEQ ID NO: 46 or 71.

237. The heteromultimer of claim 235, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 46 or 71.

238. The heteromultimer of claim 235, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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 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.

239. The heteromultimer of any one of claim 235, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456.

240. The heteromultimer of claim 235-239, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-95 of SEQ ID NO: 14.

241. The heteromultimer of claim 235-239, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22-118 of SEQ ID NO: 14.

242. The heteromultimer of claim 235-239, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

243. The heteromultimer of claim 235-239, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464.

244. A recombinant heteromultimer comprising an ALK2 polypeptide and a BMPRII polypeptide.

245. The heteromultimer of claim 244, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-123 of SEQ ID NO: 46 or 71.

246. The heteromultimer of claim 244, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 46 or 71.

247. The heteromultimer of claim 244, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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 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.

248. The heteromultimer of any one of claim 244, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456.

249. The heteromultimer of claim 244-248, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 35-99 of SEQ ID NO: 18.

250. The heteromultimer of claim 244-248, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-123 of SEQ ID NO: 18.

251. The heteromultimer of claim 244-248, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

252. The heteromultimer of claim 244-248, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466.

253. A recombinant heteromultimer comprising an ALK3 polypeptide and an BMPRII polypeptide.

254. The heteromultimer of claim 253, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-123 of SEQ ID NO: 46 or 71.

255. The heteromultimer of claim 253, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 46 or 71.

256. The heteromultimer of claim 253, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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 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.

257. The heteromultimer of any one of claim 253, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456.

258. The heteromultimer of claim 253-257, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 61-130 of SEQ ID NO: 22.

259. The heteromultimer of claim 253-257, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-152 of SEQ ID NO: 22.

260. The heteromultimer of claim 253-257, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

261. The heteromultimer of claim 253-257, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468.

262. A recombinant heteromultimer comprising an ALK4 polypeptide and a BMPRII polypeptide.

263. The heteromultimer of claim 262, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-123 of SEQ ID NO: 46 or 71.

264. The heteromultimer of claim 262, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 46 or 71.

265. The heteromultimer of claim 262, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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 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.

266. The heteromultimer of any one of claim 262, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456.

267. The heteromultimer of claim 262-266, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-101 of SEQ ID NOs: 26 or 83.

268. The heteromultimer of claim 262-266, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-126 of SEQ ID Nos: 26 or 83.

269. The heteromultimer of claim 262-266, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-34 (e.g., amino acid residues 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34) SEQ ID NO: 26 or 83, and

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.

270. The heteromultimer of claim 262-266, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470.

271. A recombinant heteromultimer comprising an ALK5 polypeptide and a BMPRII polypeptide.

272. The heteromultimer of claim 271, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-123 of SEQ ID NO: 46 or 71.

273. The heteromultimer of claim 271, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 46 or 71.

274. The heteromultimer of claim 271, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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 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.

275. The heteromultimer of any one of claim 271, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456.

276. The heteromultimer of claim 271-275, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 36-106 of SEQ ID NOs: 30 or 87.

277. The heteromultimer of claim 271-275, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-126 of SEQ ID NOs: 30 or 87.

278. The heteromultimer of claim 271-275, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

279. The heteromultimer of claim 271-275, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472.

280. A recombinant heteromultimer comprising an ALK6 polypeptide and an BMPRII polypeptide.

281. The heteromultimer of claim 280, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-123 of SEQ ID NO: 46 or 71.

282. The heteromultimer of claim 280, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 46 or 71.

283. The heteromultimer of claim 280, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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 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.

284. The heteromultimer of any one of claim 280, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456.

285. The heteromultimer of claim 280-284, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 32-102 of SEQ ID NO: 34.

286. The heteromultimer of claim 280-284, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 14-126 of SEQ ID NO: 34.

287. The heteromultimer of claim 280-284, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

288. The heteromultimer of claim 280-284, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474.

289. The heteromultimer of claim 280-284, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 62-132 of SEQ ID NO: 91.

290. The heteromultimer of claim 280-284, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 26-156 of SEQ ID NO: 91.

291. The heteromultimer of claim 280-284, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

292. A recombinant heteromultimer comprising an ALK7 polypeptide and a BMPRII polypeptide.

293. The heteromultimer of claim 280, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-123 of SEQ ID NO: 46 or 71.

294. The heteromultimer of claim 292, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 46 or 71.

295. The heteromultimer of claim 292, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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 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.

296. The heteromultimer of any one of claim 292, wherein the BMPRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 46, 47, 71, 72, 121, 123, 155, 156, 411, 412, 455, and 456.

297. The heteromultimer of claim 292-296, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NOs: 38, 305, or 309.

298. The heteromultimer of claim 292-296, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-133 of SEQ ID NOs: 38, 305, or 309.

299. The heteromultimer of claim 292-296, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 21-28 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, and 28) SEQ ID NO: 38, 305, or 309, and

b) ends at any one of amino acids 92-133 (e.g., amino acid residues 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, and 133) of SEQ ID NO: 38, 305, or 309.

300. The heteromultimer of claim 292-296, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

301. A recombinant heteromultimer comprising an ALK1 polypeptide and a TGFBRII polypeptide.

302. The heteromultimer of claim 301, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-163 of SEQ ID NO: 67.

303. The heteromultimer of claim 301, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 67.

304. The heteromultimer of claim 301, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

305. The heteromultimer of claim 301, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 42.

306. The heteromultimer of claim 301, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 42.

307. The heteromultimer of claim 301, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

308. The heteromultimer of any one of claim 301, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

309. The heteromultimer of claim 301-308, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-95 of SEQ ID NO: 14.

310. The heteromultimer of claim 301-308, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22-118 of SEQ ID NO: 14.

311. The heteromultimer of claim 301-308, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

312. The heteromultimer of claim 301-308, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464.

313. A recombinant heteromultimer comprising an ALK2 polypeptide and a TGFBRII polypeptide.

314. The heteromultimer of claim 313, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-163 of SEQ ID NO: 67.

315. The heteromultimer of claim 313, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 67.

316. The heteromultimer of claim 313, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

317. The heteromultimer of claim 313, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 42.

318. The heteromultimer of claim 313, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 42.

319. The heteromultimer of claim 313, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

320. The heteromultimer of any one of claim 313, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

321. The heteromultimer of claim 313-320, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 35-99 of SEQ ID NO: 18.

322. The heteromultimer of claim 313-320, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-123 of SEQ ID NO: 18.

323. The heteromultimer of claim 313-320, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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 313-320 (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.

324. The heteromultimer of claim 244-248, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466.

325. A recombinant heteromultimer comprising an ALK3 polypeptide and a TGFBRII polypeptide.

326. The heteromultimer of claim 325, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-163 of SEQ ID NO: 67.

327. The heteromultimer of claim 325, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 67.

328. The heteromultimer of claim 325, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

329. The heteromultimer of claim 325, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 42.

330. The heteromultimer of claim 325, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 42.

331. The heteromultimer of claim 325, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

332. The heteromultimer of any one of claim 325, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

333. The heteromultimer of claim 325-332, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 61-130 of SEQ ID NO: 22.

334. The heteromultimer of claim 325-332, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-152 of SEQ ID NO: 22.

335. The heteromultimer of claim 325-332, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

336. The heteromultimer of claim 325-332, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468.

337. A recombinant heteromultimer comprising an ALK4 polypeptide and a TGFBRII polypeptide.

338. The heteromultimer of claim 337, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-163 of SEQ ID NO: 67.

339. The heteromultimer of claim 337, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 67.

340. The heteromultimer of claim 337, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

341. The heteromultimer of claim 337, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 42.

342. The heteromultimer of claim 337, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 42.

343. The heteromultimer of claim 337, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

345. The heteromultimer of any one of claim 337, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one

of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

346. The heteromultimer of claim 337-345, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-101 of SEQ ID NOs: 26 or 83.

347. The heteromultimer of claim 337-345, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-126 of SEQ ID Nos: 26 or 83.

348. The heteromultimer of claim 337-345, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-34 (e.g., amino acid residues 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34) SEQ ID NO: 26 or 83, and

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.

349. The heteromultimer of claim 337-345, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470.

350. A recombinant heteromultimer comprising an ALK5 polypeptide and a TGFBRII polypeptide.

351. The heteromultimer of claim 350, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-163 of SEQ ID NO: 67.

352. The heteromultimer of claim 350, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 67.

353. The heteromultimer of claim 350, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

354. The heteromultimer of claim 350, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 42.

355. The heteromultimer of claim 350, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 42.

356. The heteromultimer of claim 350, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

357. The heteromultimer of any one of claim 350, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

358. The heteromultimer of claim 350-357, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 36-106 of SEQ ID NOs: 30 or 87.

359. The heteromultimer of claim 350-357, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-126 of SEQ ID NOs: 30 or 87.

360. The heteromultimer of claim 350-357, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

361. The heteromultimer of claim 350-357, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472.

362. A recombinant heteromultimer comprising an ALK6 polypeptide and a TGFBRII polypeptide.

363. The heteromultimer of claim 362, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-163 of SEQ ID NO: 67.

364. The heteromultimer of claim 362, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 67.

365. The heteromultimer of claim 362, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

366. The heteromultimer of claim 362, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 42.

367. The heteromultimer of claim 362, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 42.

368. The heteromultimer of claim 362, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

369. The heteromultimer of any one of claim 362, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

370. The heteromultimer of claim 362-369, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 32-102 of SEQ ID NO: 34.

371. The heteromultimer of claim 362-369, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 14-126 of SEQ ID NO: 34.

372. The heteromultimer of claim 362-369, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

373. The heteromultimer of claim 362-369, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474.

374. The heteromultimer of claim 362-369, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 62-132 of SEQ ID NO: 91.

375. The heteromultimer of claim 362-369, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 26-156 of SEQ ID NO: 91.

376. The heteromultimer of claim 362-369, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

377. A recombinant heteromultimer comprising an ALK7 polypeptide and a TGFBRII polypeptide.

378. The heteromultimer of claim 377, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-163 of SEQ ID NO: 67.

379. The heteromultimer of claim 377, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 67.

380. The heteromultimer of claim 377, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

381. The heteromultimer of claim 377, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 42.

382. The heteromultimer of claim 377, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 42.

383. The heteromultimer of claim 377, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 67, and

b) ends at any one of amino acids 163-191 (e.g., 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.

384. The heteromultimer of any one of claim 377, wherein the TGFBRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 42, 43, 67, 68, 127, 129, 130, 132, 157, 158, 159, 160, 415, 416, 417, 418, 459, 460, 461, and 462.

385. The heteromultimer of claim 377-384, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NOs: 38, 305, or 309.

386. The heteromultimer of claim 377-384, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-133 of SEQ ID NOs: 38, 305, or 309.

387. The heteromultimer of claim 377-384, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 21-28 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, and 28) SEQ ID NO: 38, 305, or 309, and

b) ends at any one of amino acids 92-133 (e.g., amino acid residues 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, and 133) of SEQ ID NO: 38, 305, or 309.

388. The heteromultimer of claim 377-384, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

389. A recombinant heteromultimer comprising an ALK1 polypeptide and a MISRII polypeptide.

390. The heteromultimer of claim 389, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 50, 75, or 79.

391. The heteromultimer of claim 389, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 50, 75, or 79.

392. The heteromultimer of claim 389, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

393. The heteromultimer of any one of claim 389, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458.

394. The heteromultimer of claim 389-394, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-95 of SEQ ID NO: 14.

395. The heteromultimer of claim 389-394, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22-118 of SEQ ID NO: 14.

396. The heteromultimer of claim 389-394, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

397. The heteromultimer of claim 389-394, wherein the ALK1 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 14, 15, 124, 126, 171, 172, 413, 414, 463, and 464.

398. A recombinant heteromultimer comprising an ALK2 polypeptide and a MISRII polypeptide.

399. The heteromultimer of claim 398, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 50, 75, or 79.

400. The heteromultimer of claim 398, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 50, 75, or 79.

401. The heteromultimer of claim 398, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

402. The heteromultimer of any one of claim 398, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458.

403. The heteromultimer of claim 398-402, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 35-99 of SEQ ID NO: 18.

404. The heteromultimer of claim 398-402, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-123 of SEQ ID NO: 18.

405. The heteromultimer of claim 398-402, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

406. The heteromultimer of claim 398-402, wherein the ALK2 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 18, 19, 136, 138, 173, 174, 421, 422, 465, and 466.

407. A recombinant heteromultimer comprising an ALK3 polypeptide and a MISRII polypeptide.

408. The heteromultimer of claim 407, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 50, 75, or 79.

409. The heteromultimer of claim 407, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 50, 75, or 79.

410. The heteromultimer of claim 407, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

411. The heteromultimer of any one of claim 407, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458.

412. The heteromultimer of claim 407-411, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 61-130 of SEQ ID NO: 22.

413. The heteromultimer of claim 407-411, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-152 of SEQ ID NO: 22.

414. The heteromultimer of claim 407-411, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

415. The heteromultimer of claim 407-411, wherein the ALK3 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 22, 23, 115, 117, 175, 176, 407, 408, 467, and 468.

416. A recombinant heteromultimer comprising an ALK4 polypeptide and a MISRII polypeptide.

417. The heteromultimer of claim 416, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 50, 75, or 79.

418. The heteromultimer of claim 416, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 50, 75, or 79.

419. The heteromultimer of claim 416, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

420. The heteromultimer of any one of claim 416, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458.

421. The heteromultimer of claim 416-420, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-101 of SEQ ID NOs: 26 or 83.

422. The heteromultimer of claim 416-420, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-126 of SEQ ID Nos: 26 or 83.

423. The heteromultimer of claim 416-420, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 23-34 (e.g., amino acid residues 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34) SEQ ID NO: 26 or 83, and

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.

424. The heteromultimer of claim 416-420, wherein the ALK4 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 26, 27, 83, 84, 104, 106, 177, 178, 403, 404, 469, and 470.

425. A recombinant heteromultimer comprising an ALK5 polypeptide and a MISRII polypeptide.

426. The heteromultimer of claim 425, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 50, 75, or 79.

427. The heteromultimer of claim 425, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 50, 75, or 79.

428. The heteromultimer of claim 425, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

429. The heteromultimer of any one of claim 425, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458.

430. The heteromultimer of claim 425-429, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 36-106 of SEQ ID NOs: 30 or 87.

431. The heteromultimer of claim 425-429, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-126 of SEQ ID NOs: 30 or 87.

432. The heteromultimer of claim 425-429, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

433. The heteromultimer of claim 425-429, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 30, 31, 87, 88, 139, 141, 179, 180, 423, 424, 471, and 472.

434. A recombinant heteromultimer comprising an ALK6 polypeptide and a MISRII polypeptide.

435. The heteromultimer of claim 434, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 50, 75, or 79.

436. The heteromultimer of claim 434, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 50, 75, or 79.

437. The heteromultimer of claim 434, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

438. The heteromultimer of any one of claim 434, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458.

439. The heteromultimer of claim 280-284, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 32-102 of SEQ ID NO: 34.

440. The heteromultimer of claim 434-439, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 14-126 of SEQ ID NO: 34.

441. The heteromultimer of claim 434-439, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

442. The heteromultimer of claim 434-439, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 34, 35, 91, 92, 142, 144, 181, 182, 425, 426, 473, and 474.

443. The heteromultimer of claim 434-439, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 62-132 of SEQ ID NO: 91.

444. The heteromultimer of claim 434-439, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 26-156 of SEQ ID NO: 91.

445. The heteromultimer of claim 434-439, wherein the ALK6 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

446. A recombinant heteromultimer comprising an ALK7 polypeptide and a MISRII polypeptide.

447. The heteromultimer of claim 446, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 50, 75, or 79.

448. The heteromultimer of claim 446, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 50, 75, or 79.

449. The heteromultimer of claim 446, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a 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.

450. The heteromultimer of any one of claim 446, wherein the MISRII polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 50, 51, 75, 76, 79, 80, 133, 135, 161, 162, 419, 420, 457, and 458.

451. The heteromultimer of claim 446-450, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NOs: 38, 305, or 309.

452. The heteromultimer of claim 446-450, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-133 of SEQ ID NOs: 38, 305, or 309.

453. The heteromultimer of claim 446-450, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 21-28 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, and 28) SEQ ID NO: 38, 305, or 309, and

b) ends at any one of amino acids 92-133 (e.g., amino acid residues 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, and 133) of SEQ ID NO: 38, 305, or 309.

454. The heteromultimer of claim 446-450, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 38, 39, 301, 302, 305, 306, 309, 310, 313, 112, 114, 183, 184, 405, 406, 475, and 476.

455. A recombinant heteromultimer comprising an ALK1 polypeptide and at least one additional TGF-beta type I receptor polypeptide, wherein the at least one additional TGF-beta type I receptor polypeptide is not an ALK1 polypeptide.

456. The heteromultimer of claim 455, wherein the ALK1 polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK1 sequence (e.g., SEQ ID NO) disclosed herein.

457. The heteromultimer of claim 455 or 456, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK1 polypeptide is selected from the group

consisting of: an ALK2 polypeptide, an ALK3 polypeptide, an ALK4 polypeptide, an ALK5 polypeptide, an ALK6 polypeptide, and an ALK7 polypeptide.

458. The heteromultimer of claim 457, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK1 polypeptide is selected from the group consisting of:

a) an ALK2 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK2 sequence (e.g., SEQ ID NO) disclosed herein,

b) an ALK3 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK3 sequence (e.g., SEQ ID NO) disclosed herein,

c) an ALK4 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK4 sequence (e.g., SEQ ID NO) disclosed herein,

d) an ALK5 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK5 sequence (e.g., SEQ ID NO) disclosed herein,

e) an ALK6 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK6 sequence (e.g., SEQ ID NO) disclosed herein,

f) an ALK7 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK7 sequence (e.g., SEQ ID NO) disclosed herein.

459. A recombinant heteromultimer comprising an ALK2 polypeptide and at least one additional TGF-beta type I receptor polypeptide, wherein the at least one additional TGF-beta type I receptor polypeptide is not an ALK2 polypeptide.

460. The heteromultimer of claim 459, wherein the ALK2 polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK2 sequence (e.g., SEQ ID NO) disclosed herein.

461. The heteromultimer of claim 459 or 460, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK2 polypeptide is selected from the group consisting of: an ALK3 polypeptide, an ALK4 polypeptide, an ALK5 polypeptide, an ALK6 polypeptide, and an ALK7 polypeptide.

462. The heteromultimer of claim 461, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK2 polypeptide is selected from the group consisting of:

a) an ALK3 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK3 sequence (e.g., SEQ ID NO) disclosed herein,

b) an ALK4 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK4 sequence (e.g., SEQ ID NO) disclosed herein,

c) an ALK5 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK5 sequence (e.g., SEQ ID NO) disclosed herein,

d) an ALK6 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK6 sequence (e.g., SEQ ID NO) disclosed herein,

e) an ALK7 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK7 sequence (e.g., SEQ ID NO) disclosed herein.

463. A recombinant heteromultimer comprising an ALK3 polypeptide and at least one additional TGF-beta type I receptor polypeptide, wherein the at least one additional TGF-beta type I receptor polypeptide is not an ALK3 polypeptide.

464. The heteromultimer of claim 463, wherein the ALK3 polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK3 sequence (e.g., SEQ ID NO) disclosed herein.

465. The heteromultimer of claim 463 or 464, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK3 polypeptide is selected from the group consisting of: an ALK4 polypeptide, an ALK5 polypeptide, an ALK6 polypeptide, and an ALK7 polypeptide.

466. The heteromultimer of claim 465, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK3 polypeptide is selected from the group consisting of:

a) an ALK4 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK4 sequence (e.g., SEQ ID NO) disclosed herein,

b) an ALK5 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK5 sequence (e.g., SEQ ID NO) disclosed herein,

c) an ALK6 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK6 sequence (e.g., SEQ ID NO) disclosed herein,

d) an ALK7 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK7 sequence (e.g., SEQ ID NO) disclosed herein.

467. A recombinant heteromultimer comprising an ALK4 polypeptide and at least one additional TGF-beta type I receptor polypeptide, wherein the at least one additional TGF-beta type I receptor polypeptide is not an ALK4 polypeptide.

468. The heteromultimer of claim 467, wherein the ALK4 polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK4 sequence (e.g., SEQ ID NO) disclosed herein.

469. The heteromultimer of claim 467 or 468, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK4 polypeptide is selected from the group consisting of: an ALK5 polypeptide, an ALK6 polypeptide, and an ALK7 polypeptide.

470. The heteromultimer of claim 469, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK4 polypeptide is selected from the group consisting of:

a) an ALK5 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK5 sequence (e.g., SEQ ID NO) disclosed herein,

b) an ALK6 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK6 sequence (e.g., SEQ ID NO) disclosed herein,

c) an ALK7 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK7 sequence (e.g., SEQ ID NO) disclosed herein.

471. A recombinant heteromultimer comprising an ALK5 polypeptide and at least one additional TGF-beta type I receptor polypeptide, wherein the at least one additional TGF-beta type I receptor polypeptide is not an ALK5 polypeptide.

472. The heteromultimer of claim 471, wherein the ALK5 polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK5 sequence (e.g., SEQ ID NO) disclosed herein.

473. The heteromultimer of claim 471 or 472, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK5 polypeptide is selected from the group consisting of: an ALK6 polypeptide, and an ALK7 polypeptide.

474. The heteromultimer of claim 473, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK5 polypeptide is selected from the group consisting of:

a) an ALK6 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK6 sequence (e.g., SEQ ID NO) disclosed herein,

b) an ALK7 polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK7 sequence (e.g., SEQ ID NO) disclosed herein.

475. A recombinant heteromultimer comprising an ALK6 polypeptide and at least one additional TGF-beta type I receptor polypeptide, wherein the at least one additional TGF-beta type I receptor polypeptide is not an ALK6 polypeptide.

476. The heteromultimer of claim 475, wherein the ALK6 polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK6 sequence (e.g., SEQ ID NO) disclosed herein.

477. The heteromultimer of claim 475 or 476, wherein the least one additional TGF-beta type I receptor polypeptide that is not an ALK6 polypeptide is an ALK7 polypeptide.

478. The heteromultimer of claim 477, ALK7 polypeptide an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ALK7 sequence (e.g., SEQ ID NO) disclosed herein.

479. A recombinant heteromultimer comprising an ActRIIA polypeptide and at least one additional TGF-beta type II receptor polypeptide, wherein the at least one additional TGF-beta type II receptor polypeptide is not an ActRIIA polypeptide.

480. The heteromultimer of claim 479, wherein the ActRIIA polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%. 98%, 99%, or 100% identical to an ActRIIA sequence (e.g., SEQ ID NO) disclosed herein.

481. The heteromultimer of claim 479 or 480, wherein the least one additional TGF-beta type II receptor polypeptide that is not an ActRIIA polypeptide is selected from the group consisting of: an ActRIIB polypeptide, a BMPRII polypeptide, a TGFBRII polypeptide, and an MISRII polypeptide.

482. The heteromultimer of claim 481, wherein the least one additional TGF-beta type II receptor polypeptide that is not an ActRIIA polypeptide is selected from the group consisting of:

a) an ActRIIB polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ActRIIB sequence (e.g., SEQ ID NO) disclosed herein,

b) an BMPRII polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an BMPRII sequence (e.g., SEQ ID NO) disclosed herein,

c) an TGFBRII polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an TGFBRII sequence (e.g., SEQ ID NO) disclosed herein,

d) an MISRII polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an MISRII sequence (e.g., SEQ ID NO) disclosed herein.

483. A recombinant heteromultimer comprising an ActRIIB polypeptide and at least one additional TGF-beta type II receptor polypeptide, wherein the at least one additional TGF-beta type II receptor polypeptide is not an ActRIIB polypeptide.

484. The heteromultimer of claim 483, wherein the ActRIIB polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an ActRIIB sequence (e.g., SEQ ID NO) disclosed herein.

485. The heteromultimer of claim 483 or 484, wherein the least one additional TGF-beta type II receptor polypeptide that is not an ActRIIB polypeptide is selected from the group consisting of: a BMPRII polypeptide, a TGFBRII polypeptide, and an MISRII polypeptide.

486. The heteromultimer of claim 485, wherein the least one additional TGF-beta type II receptor polypeptide that is not an ActRIIB polypeptide is selected from the group consisting of:

b) an BMPRII polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an BMPRII sequence (e.g., SEQ ID NO) disclosed herein,

c) an TGFBRII polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an TGFBRII sequence (e.g., SEQ ID NO) disclosed herein,

d) an MISRII polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an MISRII sequence (e.g., SEQ ID NO) disclosed herein.

487. A recombinant heteromultimer comprising an BMPRII polypeptide and at least one additional TGF-beta type II receptor polypeptide, wherein the at least one additional TGF-beta type II receptor polypeptide is not an BMPRII polypeptide.

488. The heteromultimer of claim 487, wherein the BMPRII polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an BMPRII sequence (e.g., SEQ ID NO) disclosed herein.

489. The heteromultimer of claim 487or 488, wherein the least one additional TGF-beta type II receptor polypeptide that is not an BMPRII polypeptide is selected from the group consisting of: a TGFBRII polypeptide, and an MISRII polypeptide.

490. The heteromultimer of claim 489, wherein the least one additional TGF-beta type II receptor polypeptide that is not an BMPRII polypeptide is selected from the group consisting of:

a) an TGFBRII polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an TGFBRII sequence (e.g., SEQ ID NO) disclosed herein,

b) an MISRII polypeptide that comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an MISRII sequence (e.g., SEQ ID NO) disclosed herein.

491. A recombinant heteromultimer comprising a TGFBRII polypeptide and at least one additional TGF-beta type II receptor polypeptide, wherein the at least one additional TGF-beta type II receptor polypeptide is not an TGFBRII polypeptide.

492. The heteromultimer of claim 491, wherein the TGFBRII polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an TGFBRII sequence (e.g., SEQ ID NO) disclosed herein.

493. The heteromultimer of claim 491 or 492, wherein the least one additional TGF-beta type II receptor polypeptide that is not an TGFBRII polypeptide is an MISRII polypeptide.

494. The heteromultimer of claim 493, wherein the MISRII polypeptide comprises an amino acids sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an MISRII sequence (e.g., SEQ ID NO) disclosed herein.

495. A recombinant heteromultimer as described in the table: "Type II:Type II Receptor Heterodimers".

460. A recombinant heteromultimer as described in the table: "Type I:Type I Receptor Heterodimers".

461. A recombinant heteromultimer as described in the table: "Type I:Type II Receptor Heterodimers".

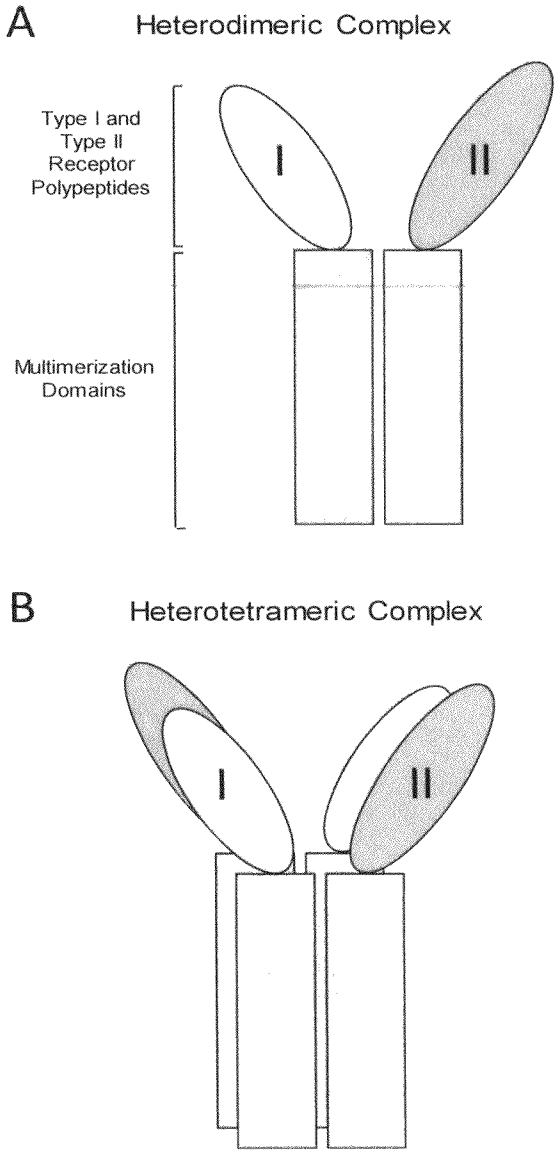

A
Heterodimeric Complex
Type I and
Type II
Receptor
Polypeptides
I
II
Multimerization
Domains
B
Heterotetrameric Complex
I
II

FIGURE 1

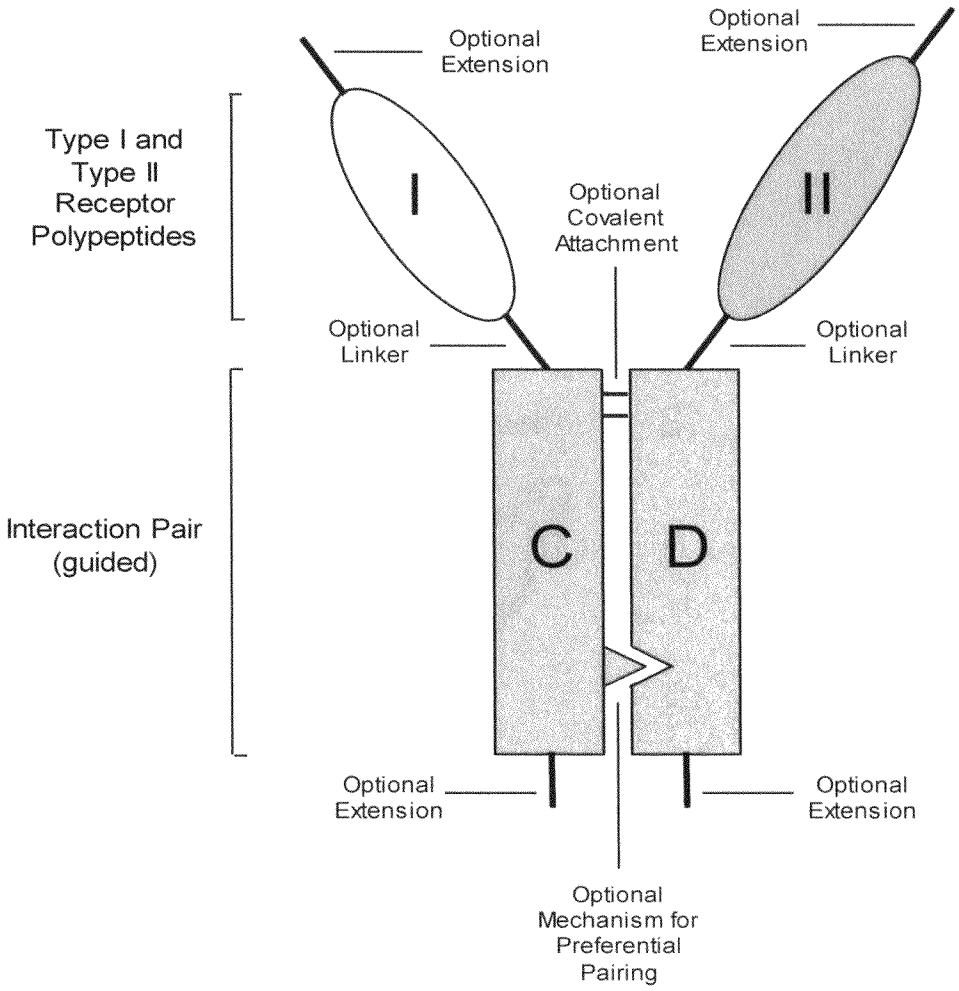

Schematic Heteromeric Protein Complex
Optional Extension
Optional Extension
Type I and Type II Receptor Polypeptides
I
II
Optional Covalent Attachment
Optional Linker
Optional Linker
Interaction Pair (guided)
C
D
Optional Extension
Optional Extension
Optional Mechanism for Preferential Pairing

FIGURE 2

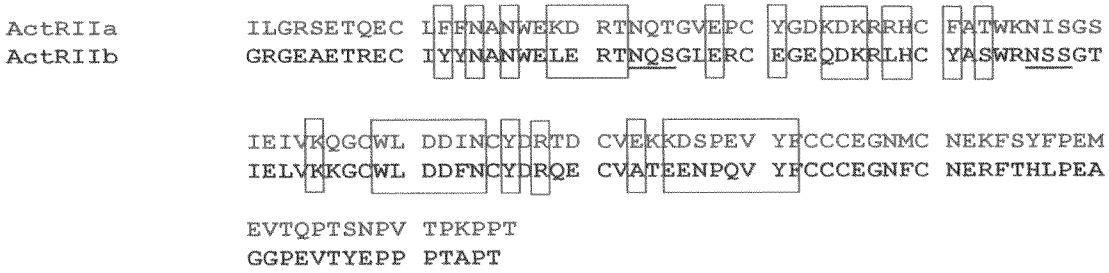

ActRIIa ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
ActRIIb GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
EVTQPTSNPV TPKPPT
GGPEVTYEPP PTAPT

FIGURE 3

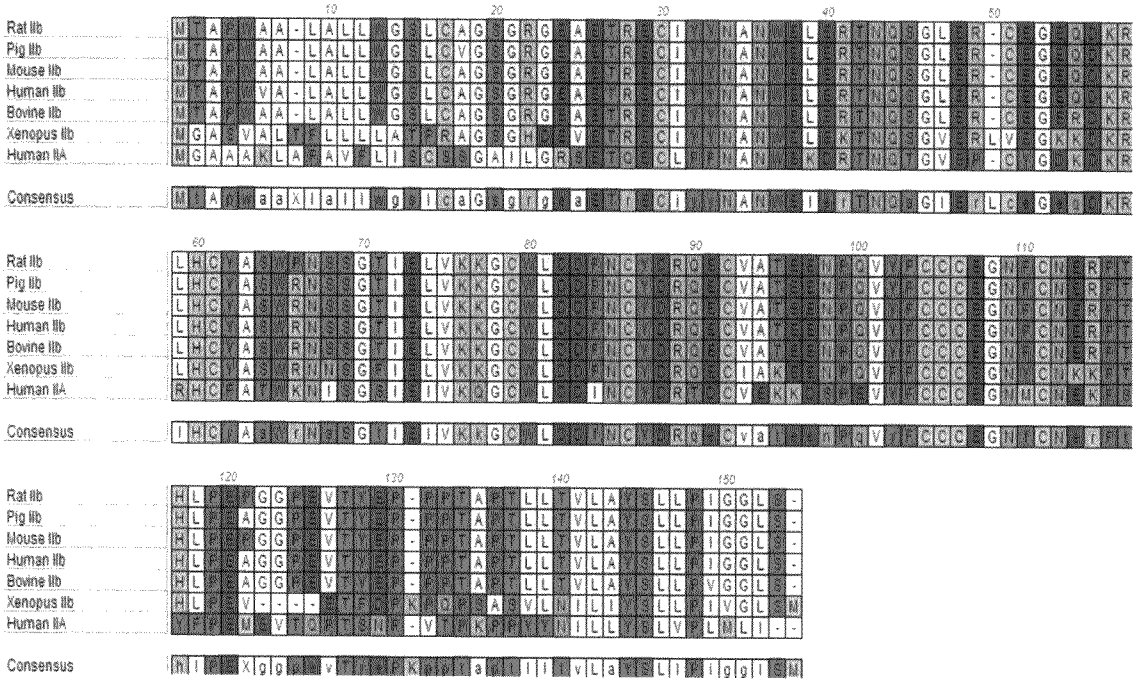

Rat IIb
Pig IIb
Mouse IIb
Human IIb
Bovine IIb
Xenopus IIb
Human IIA
Consensus

FIGURE 4

```
IgG1   -------THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF 53
IgG4   ---ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF 57
IgG2   --------VECPPCPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 51
IgG3   EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 60
                  ** **** . * ********************************:*****:*

IgG1   NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 113
IgG4   NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT 117
IgG2   NWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT 111
IgG3   KWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 120
       :*******************:***:********:*****************.**:.****

IgG1   ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 173
IgG4   ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 177
IgG2   ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 171
IgG3   ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTP 180
       ***:***************:*****************************.*******:***

IgG1   PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 225
IgG4   PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK 229
IgG2   PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 223
IgG3   PMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK 232
       *:***********:*********:**:************::******** **
```

FIGURE 5

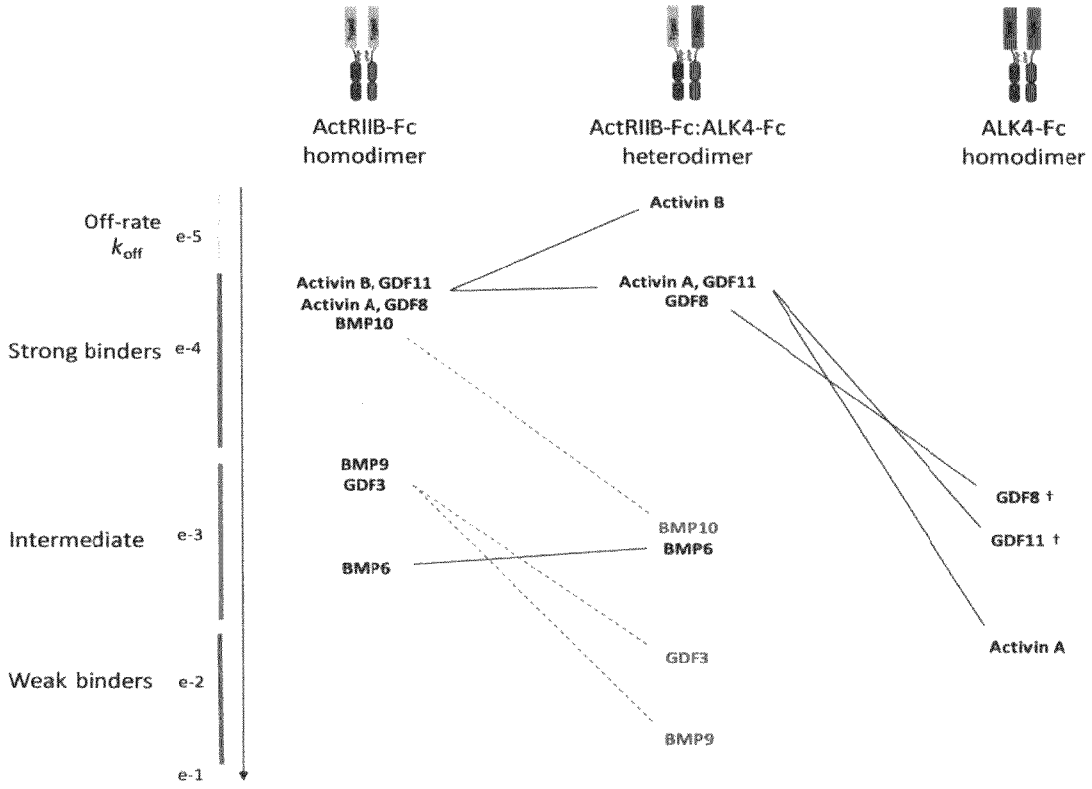

ActRIIB-Fc homodimer
ActRIIB-Fc:ALK4-Fc heterodimer
ALK4-Fc homodimer
Off-rate k_{off}
e-5
Strong binders
e-4
Intermediate
e-3
Weak binders
e-2
e-1
Activin B
Activin B, GDF11
Activin A, GDF8
BMP10
Activin A, GDF11
GDF8
BMP9
GDF3
BMP10
BMP6
BMP6
GDF3
BMP9
GDF8 †
GDF11 †
Activin A

FIGURE 6

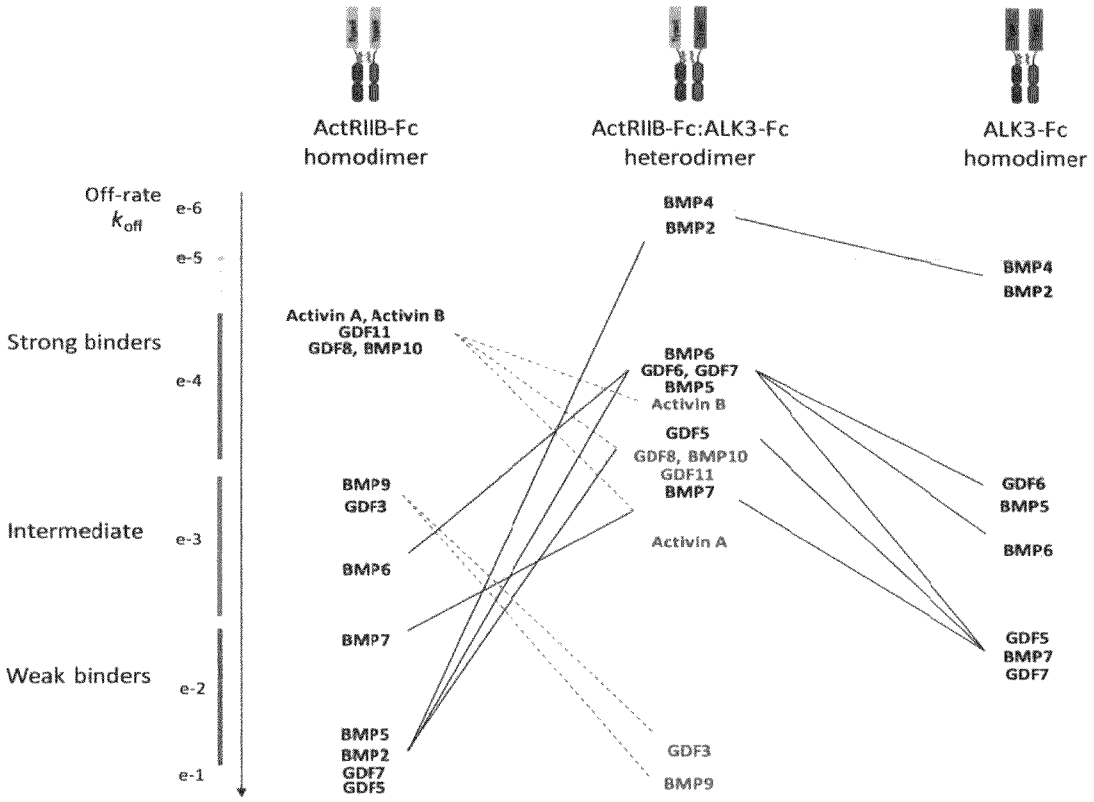

ActRIIB-Fc homodimer
ActRIIB-Fc:ALK3-Fc heterodimer
ALK3-Fc homodimer
Off-rate k_{off}
e-6
e-5
e-4
e-3
e-2
e-1
Strong binders
Intermediate
Weak binders
Activin A, Activin B GDF11 GDF8, BMP10
BMP9 GDF3
BMP6
BMP7
BMP5 BMP2 GDF7 GDF5
BMP4 BMP2
BMP6 GDF6, GDF7 BMP5 Activin B
GDF5
GDF8, BMP10 GDF11 BMP7
Activin A
GDF3
BMP9
BMP4 BMP2
GDF6 BMP5
BMP6
GDF5 BMP7 GDF7

FIGURE 7

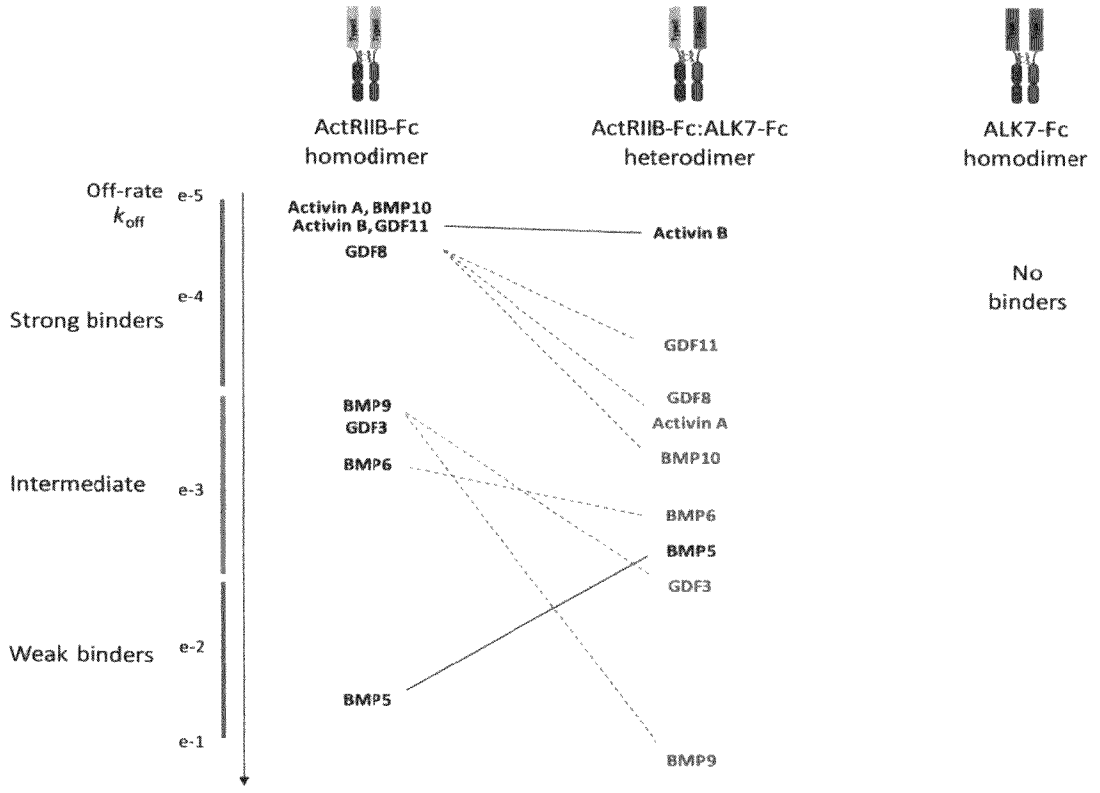

ActRIIB-Fc homodimer
ActRIIB-Fc:ALK7-Fc heterodimer
ALK7-Fc homodimer
Off-rate k_{off}
e-5
e-4
e-3
e-2
e-1
Strong binders
Intermediate
Weak binders
Activin A, BMP10
Activin B, GDF11
GDF8
BMP9
GDF3
BMP6
BMP5
Activin B
GDF11
GDF8
Activin A
BMP10
BMP6
BMP5
GDF3
BMP9
No binders

FIGURE 8

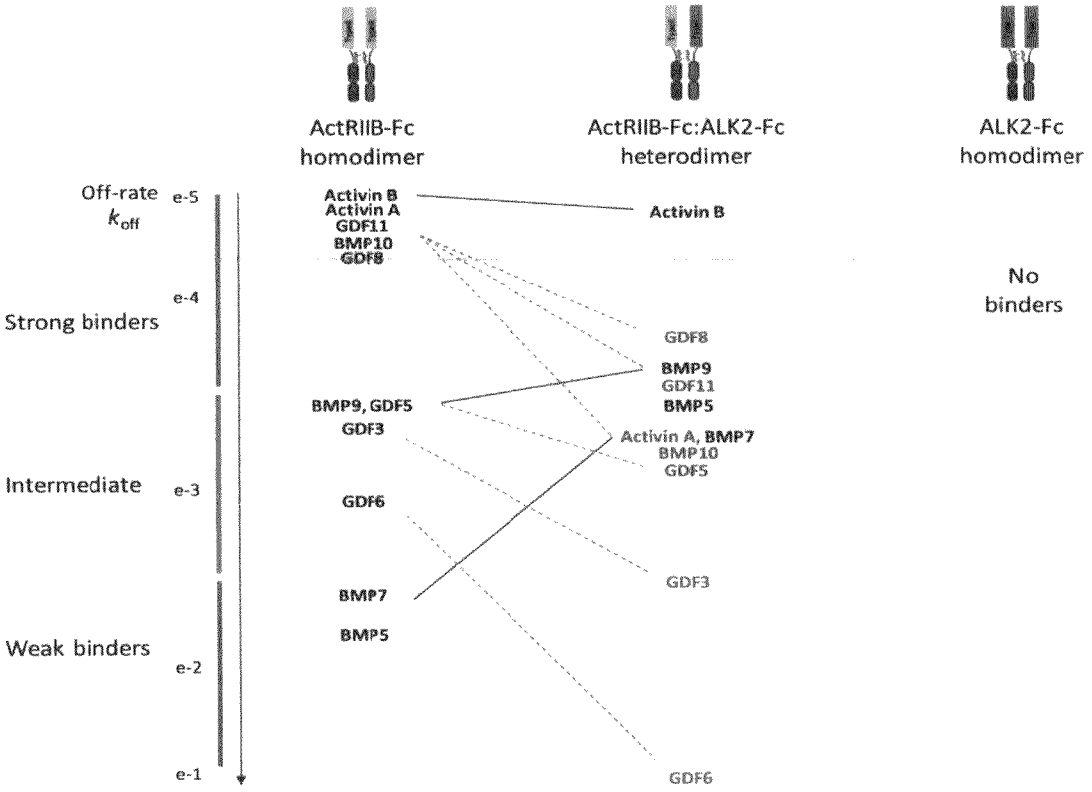

ActRIIB-Fc homodimer
ActRIIB-Fc:ALK2-Fc heterodimer
ALK2-Fc homodimer
Off-rate k_{off}
e-5
e-4
e-3
e-2
e-1
Strong binders
Intermediate
Weak binders
Activin B
Activin A
GDF11
BMP10
GDF8
BMP9, GDF5
GDF3
GDF6
BMP7
BMP5
Activin B
GDF8
BMP9
GDF11
BMP5
Activin A, BMP7
BMP10
GDF5
GDF3
GDF6
No binders

FIGURE 9

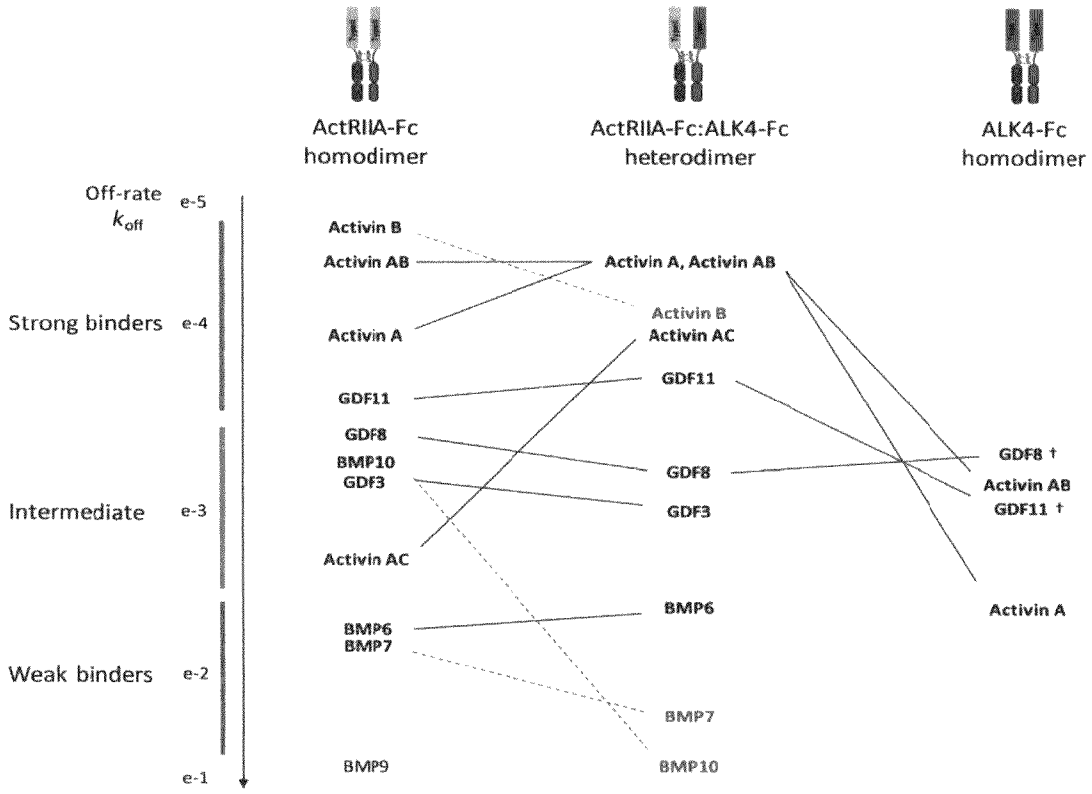

ActRIIA-Fc homodimer
ActRIIA-Fc:ALK4-Fc heterodimer
ALK4-Fc homodimer
Off-rate k_{off}
e-5
Strong binders
e-4
Intermediate
e-3
Weak binders
e-2
e-1
Activin B
Activin AB
Activin A
GDF11
GDF8
BMP10
GDF3
Activin AC
BMP6
BMP7
BMP9
Activin A, Activin AB
Activin B
Activin AC
GDF11
GDF8
GDF3
BMP6
BMP7
BMP10
GDF8 †
Activin AB
GDF11 †
Activin A

FIGURE 10

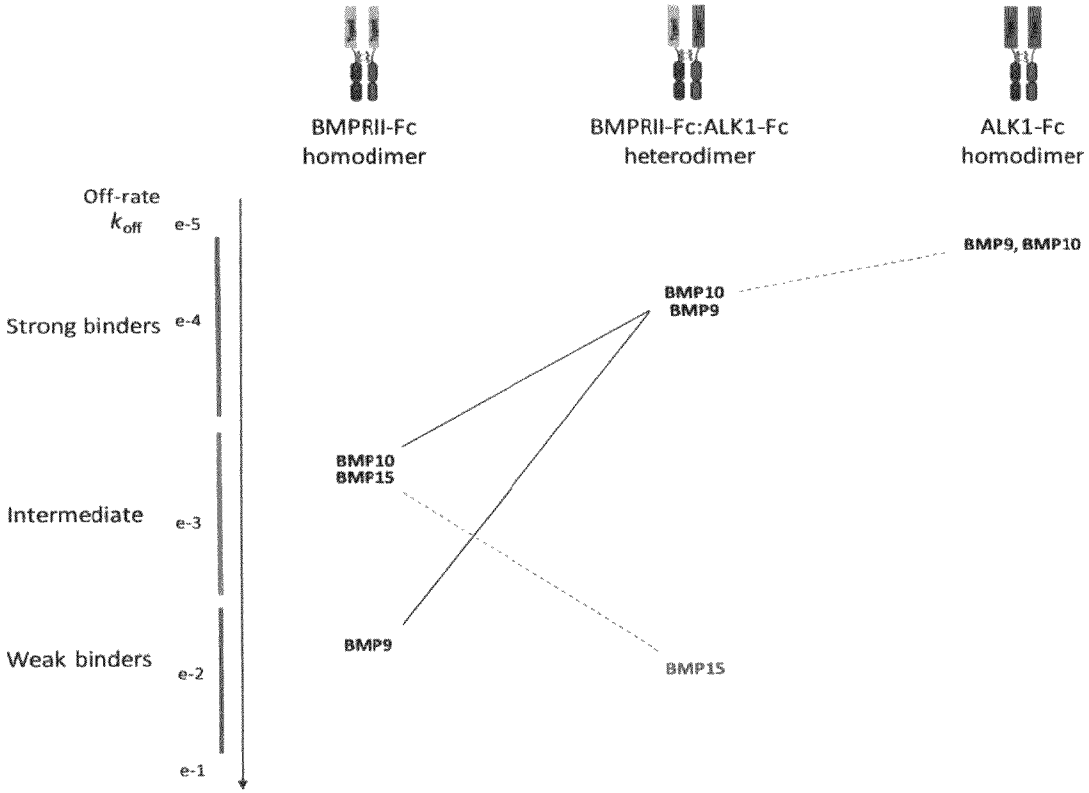

BMPRII-Fc
homodimer
BMPRII-Fc:ALK1-Fc
heterodimer
ALK1-Fc
homodimer
Off-rate
k_{off}
e-5
Strong binders
e-4
Intermediate
e-3
Weak binders
e-2
e-1
BMP9, BMP10
BMP10
BMP9
BMP10
BMP15
BMP9
BMP15

FIGURE 11

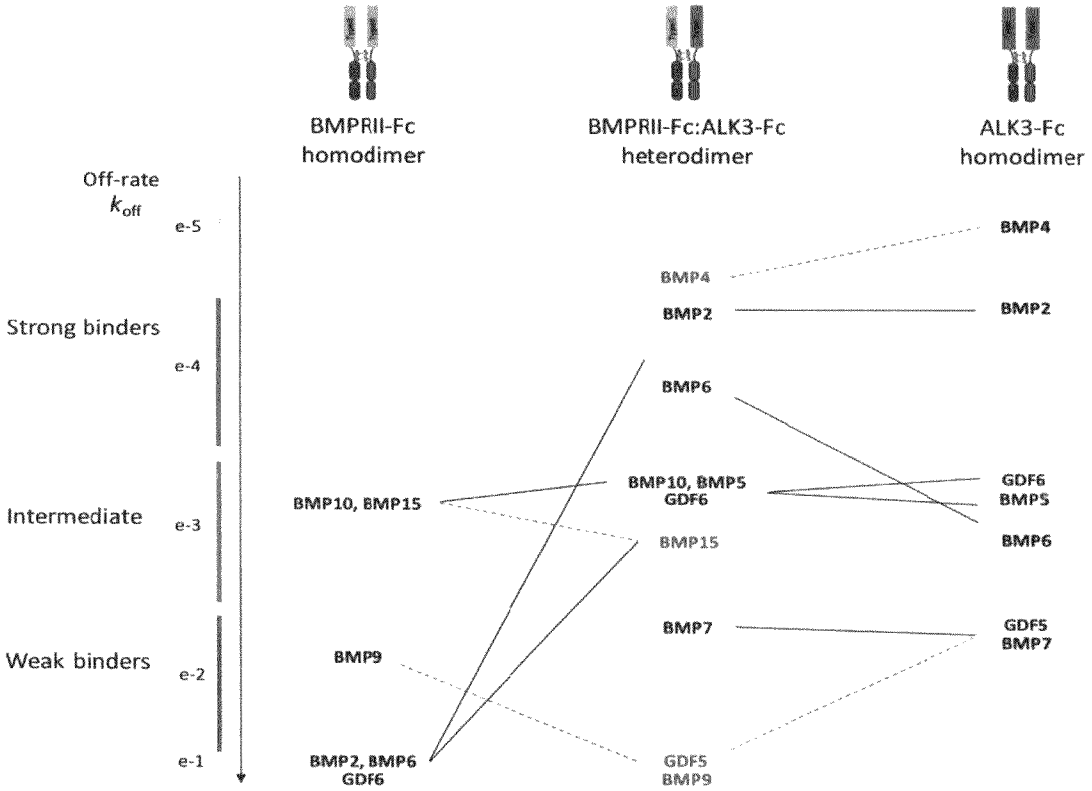

BMPRII-Fc homodimer
BMPRII-Fc:ALK3-Fc heterodimer
ALK3-Fc homodimer
Off-rate k_{off}
e-5
e-4
e-3
e-2
e-1
Strong binders
Intermediate
Weak binders
BMP10, BMP15
BMP9
BMP2, BMP6 GDF6
BMP4
BMP2
BMP6
BMP10, BMP5 GDF6
BMP15
BMP7
GDF5 BMP9
BMP4
BMP2
GDF6 BMP5
BMP6
GDF5 BMP7

FIGURE 12

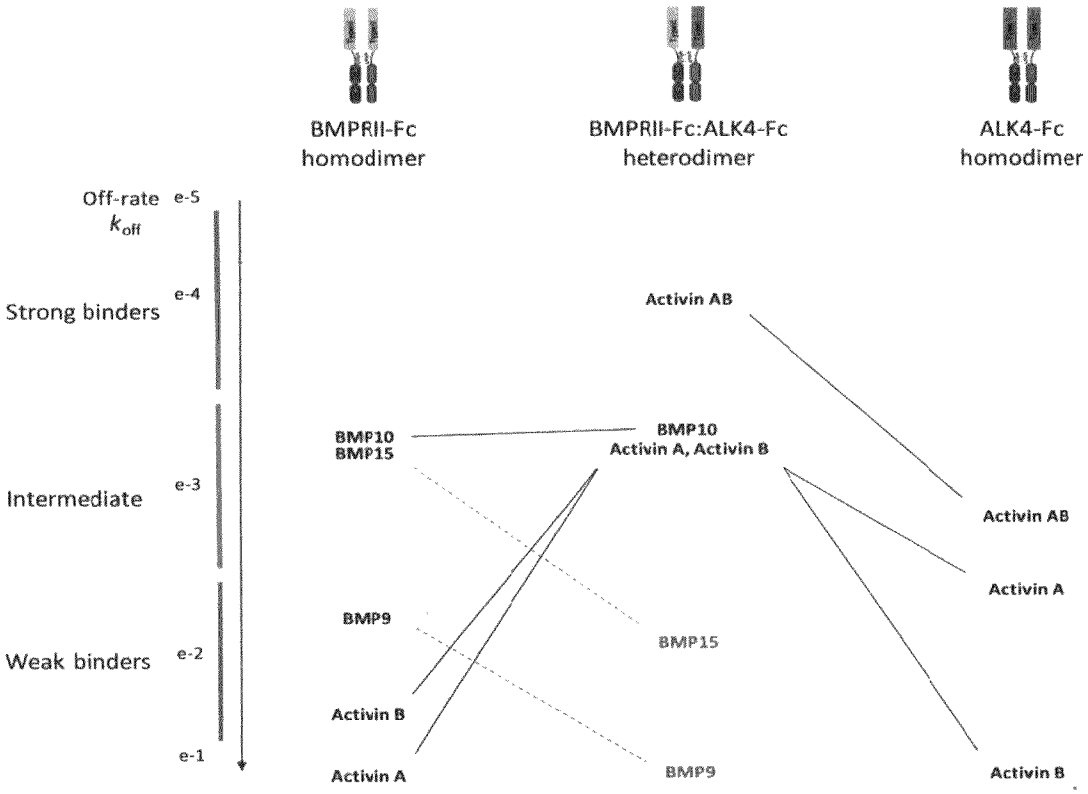

BMPRII-Fc
homodimer
BMPRII-Fc:ALK4-Fc
heterodimer
ALK4-Fc
homodimer
Off-rate
k_{off}
e-5
e-4
e-3
e-2
e-1
Strong binders
Intermediate
Weak binders
Activin AB
BMP10
BMP15
BMP10
Activin A, Activin B
Activin AB
Activin A
BMP9
BMP15
Activin B
BMP9
Activin A
Activin B

FIGURE 13

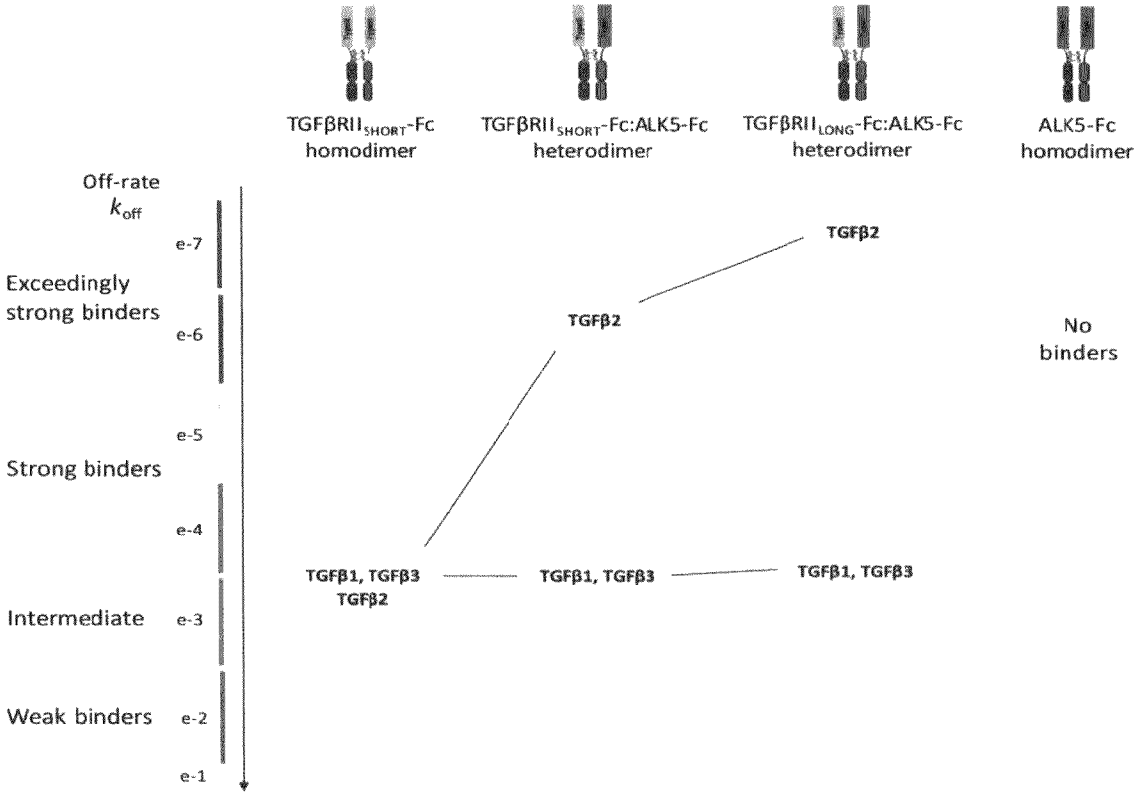

TGFβRII$_{SHORT}$-Fc homodimer
TGFβRII$_{SHORT}$-Fc:ALK5-Fc heterodimer
TGFβRII$_{LONG}$-Fc:ALK5-Fc heterodimer
ALK5-Fc homodimer
Off-rate k_{off}
e-7
Exceedingly strong binders
e-6
e-5
Strong binders
e-4
e-3
Intermediate
Weak binders
e-2
e-1
TGFβ2
TGFβ2
No binders
TGFβ1, TGFβ3
TGFβ2
TGFβ1, TGFβ3
TGFβ1, TGFβ3

FIGURE 14

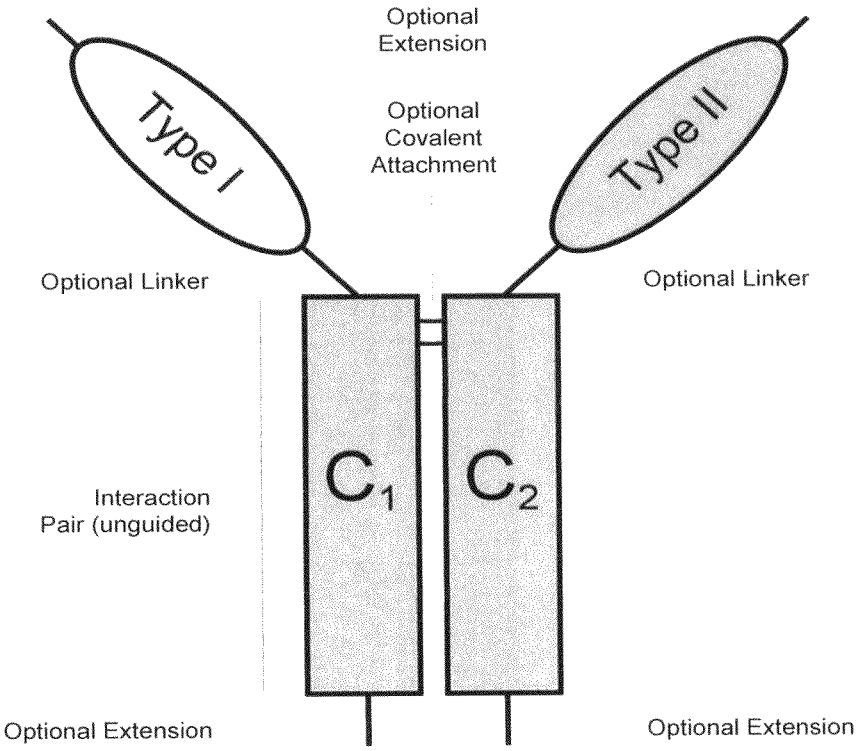

Optional
Extension
Optional
Covalent
Attachment
Type I
Type II
Optional Linker
Optional Linker
Interaction
Pair (unguided)
C1
C2
Optional Extension
Optional Extension

FIGURE 15A

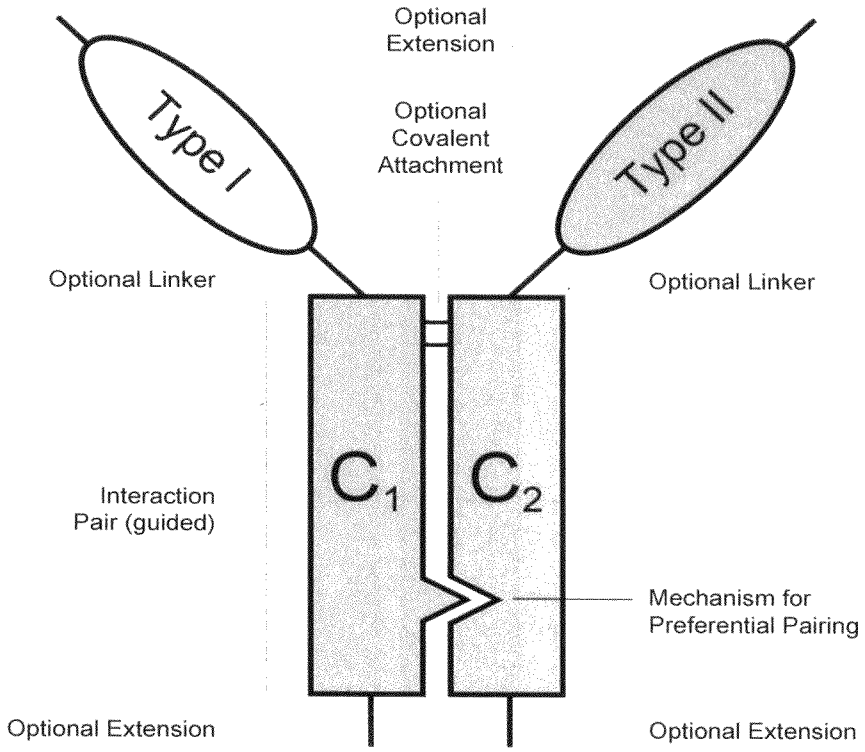

Optional
Extension
Optional
Covalent
Attachment
Type I
Type II
Optional Linker
Optional Linker
C1
C2
Interaction
Pair (guided)
Mechanism for
Preferential Pairing
Optional Extension
Optional Extension

FIGURE 15B

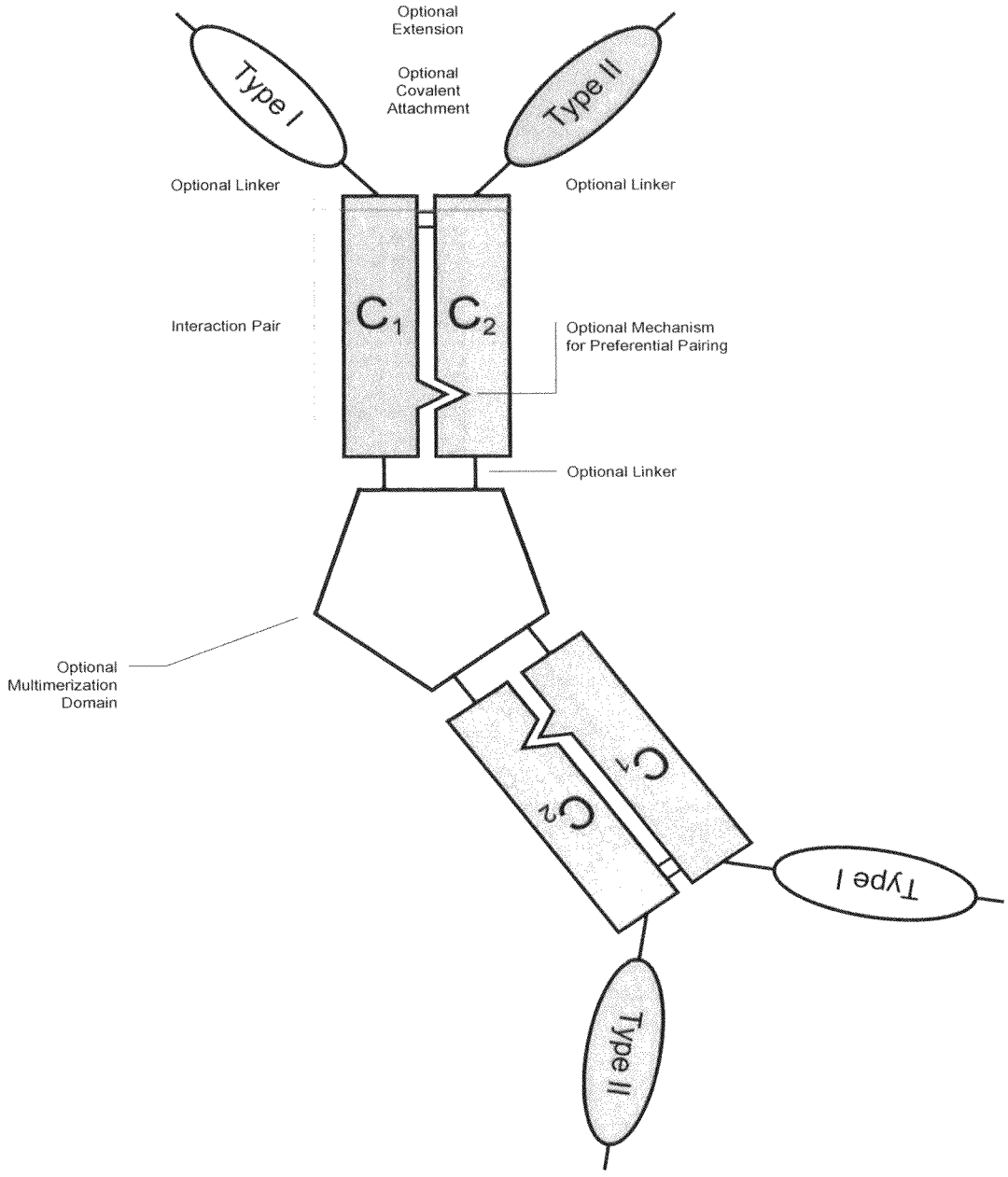

Optional Extension
Optional Covalent Attachment
Type I
Type II
Optional Linker
Optional Linker
Interaction Pair
C1
C2
Optional Mechanism for Preferential Pairing
Optional Linker
Optional Multimerization Domain
C1
C2
Type I
Type II

FIGURE 15C

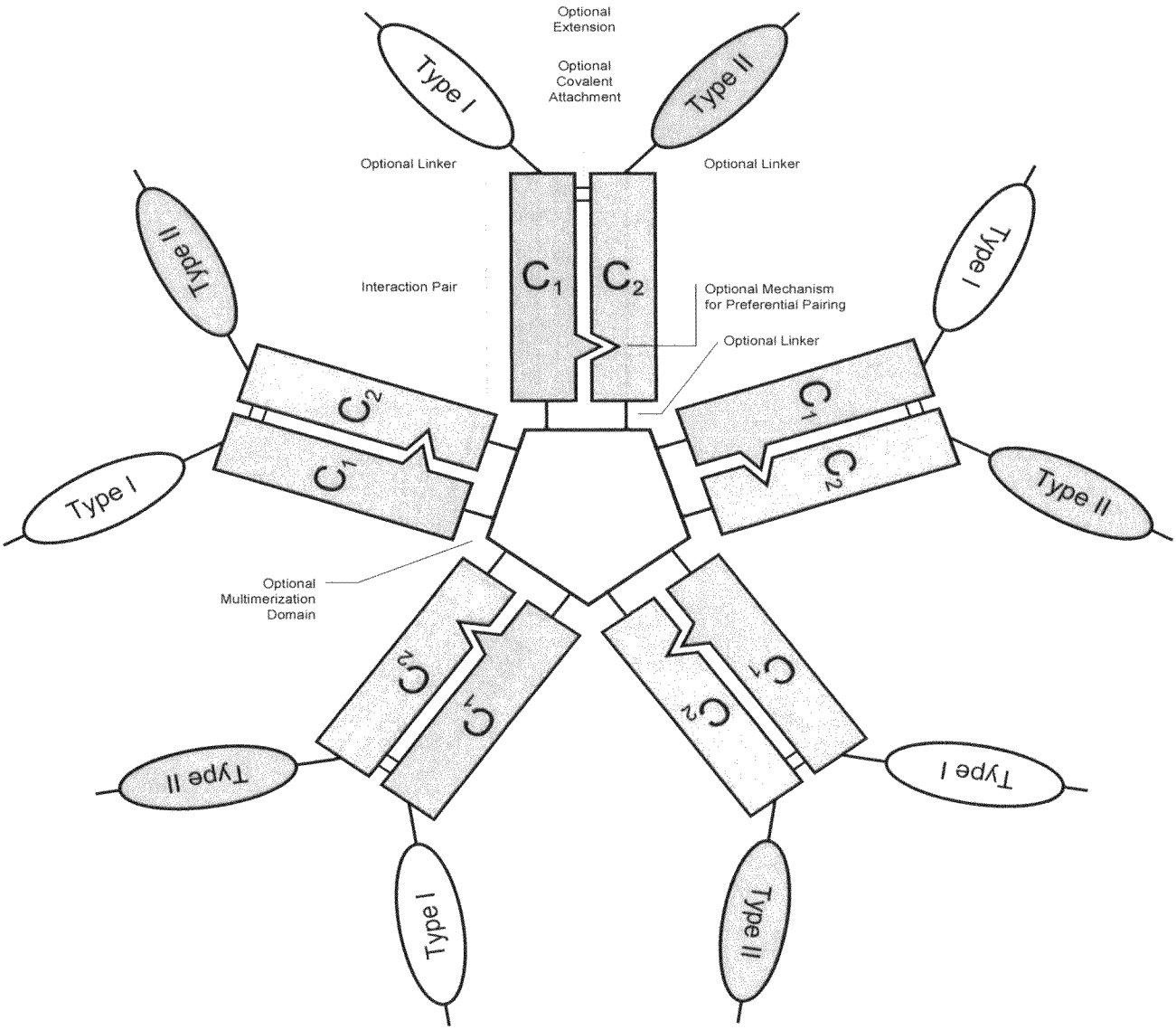

Optional Extension
Optional Covalent Attachment
Type I
Type II
Optional Linker
Optional Linker
Interaction Pair
C1
C2
Optional Mechanism for Preferential Pairing
Optional Linker
Optional Multimerization Domain

FIGURE 15D

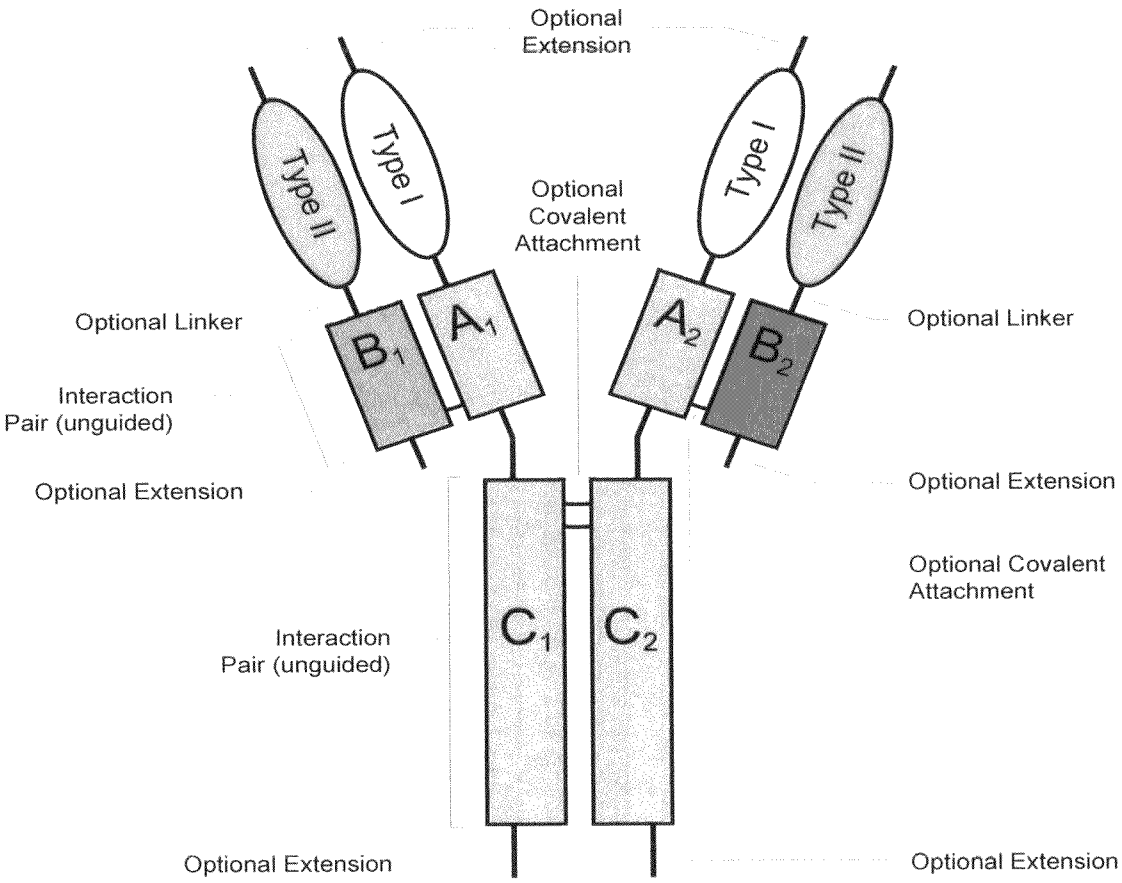

Optional Extension
Type II
Type I
Optional Covalent Attachment
Type I
Type II
Optional Linker
B_1
A_1
A_2
B_2
Optional Linker
Interaction Pair (unguided)
Optional Extension
Optional Extension
Optional Covalent Attachment
Interaction Pair (unguided)
C_1
C_2
Optional Extension
Optional Extension

FIGURE 16A

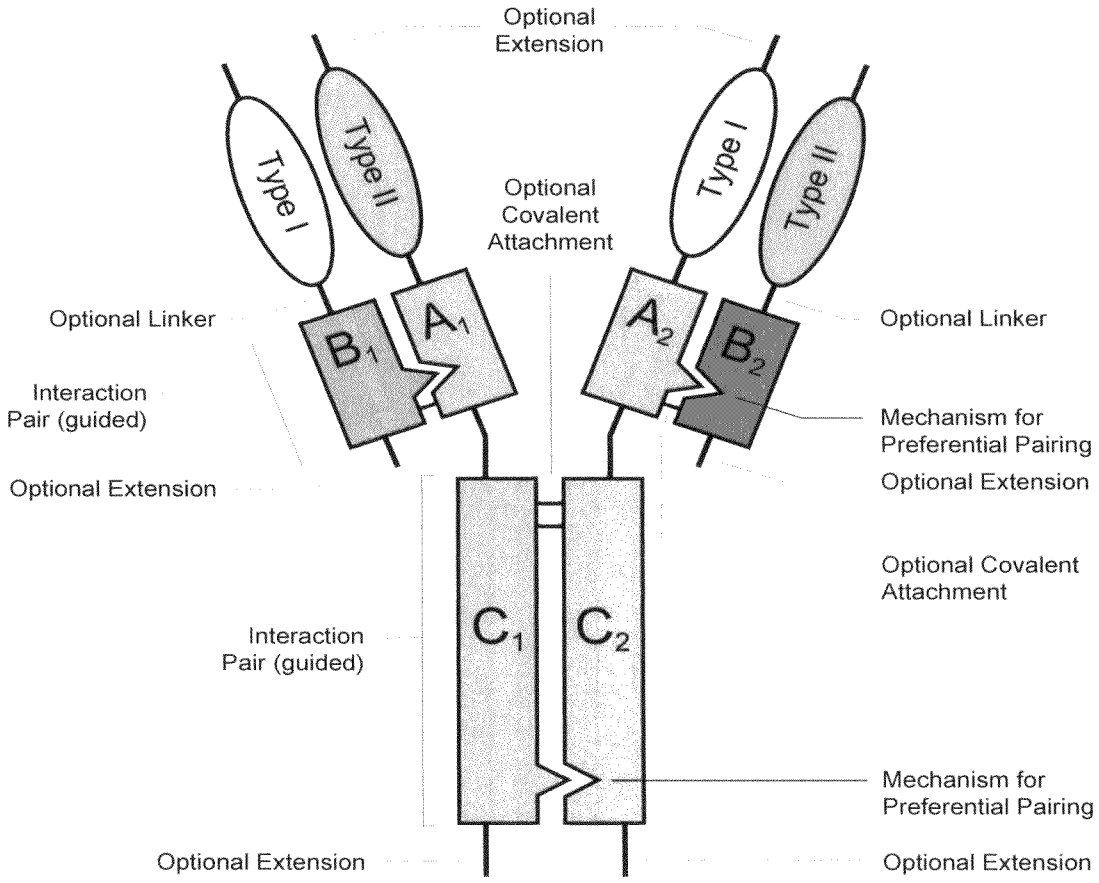

Optional Extension
Type I
Type II
Type I
Type II
Optional Covalent Attachment
Optional Linker
Optional Linker
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Extension
Optional Extension
Optional Covalent Attachment
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 16B

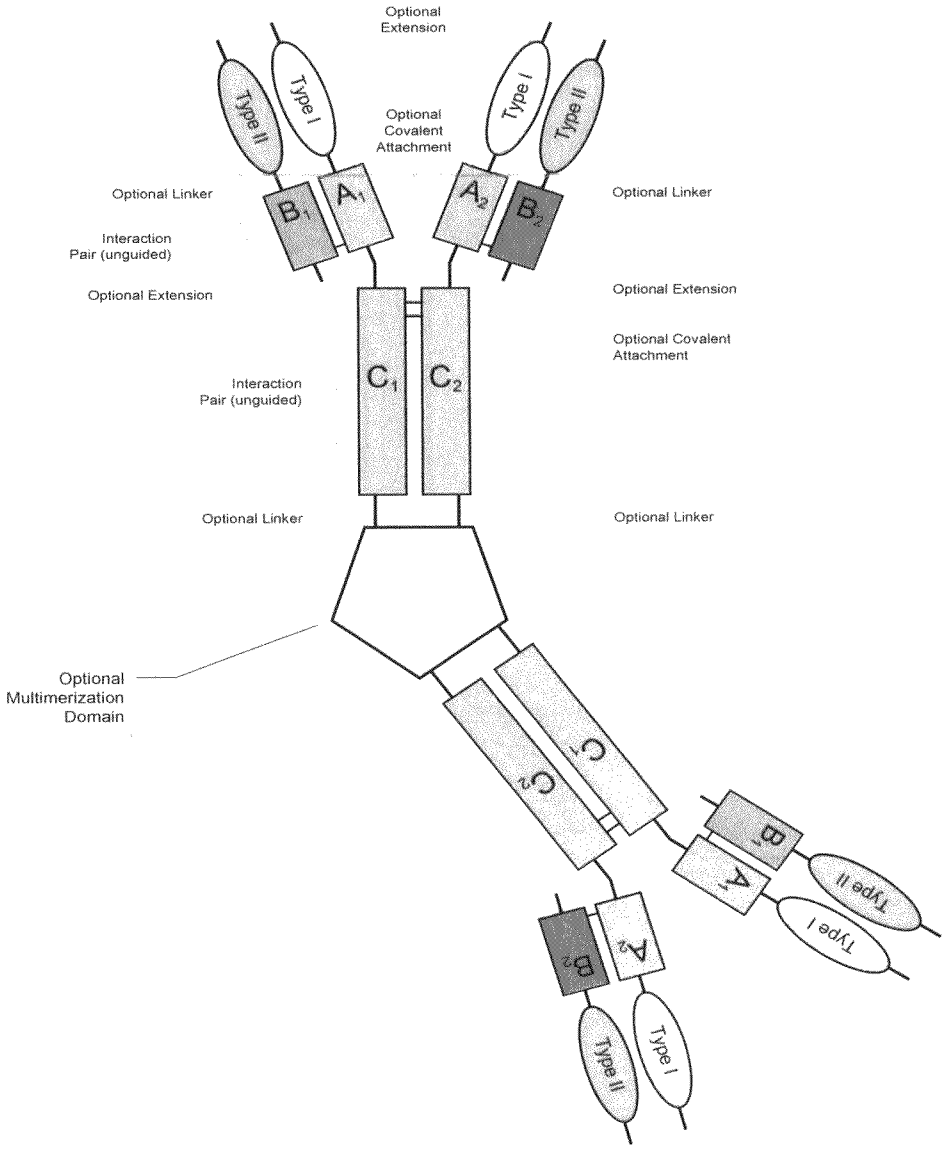

Optional Extension
Type II
Type I
Optional Covalent Attachment
Type I
Type II
Optional Linker
B1
A1
A2
B2
Optional Linker
Interaction Pair (unguided)
Optional Extension
Optional Extension
Optional Covalent Attachment
C1
C2
Interaction Pair (unguided)
Optional Linker
Optional Linker
Optional Multimerization Domain
C1
C2
B1
A1
Type II
Type I
B2
A2
Type II
Type I

FIGURE 16C

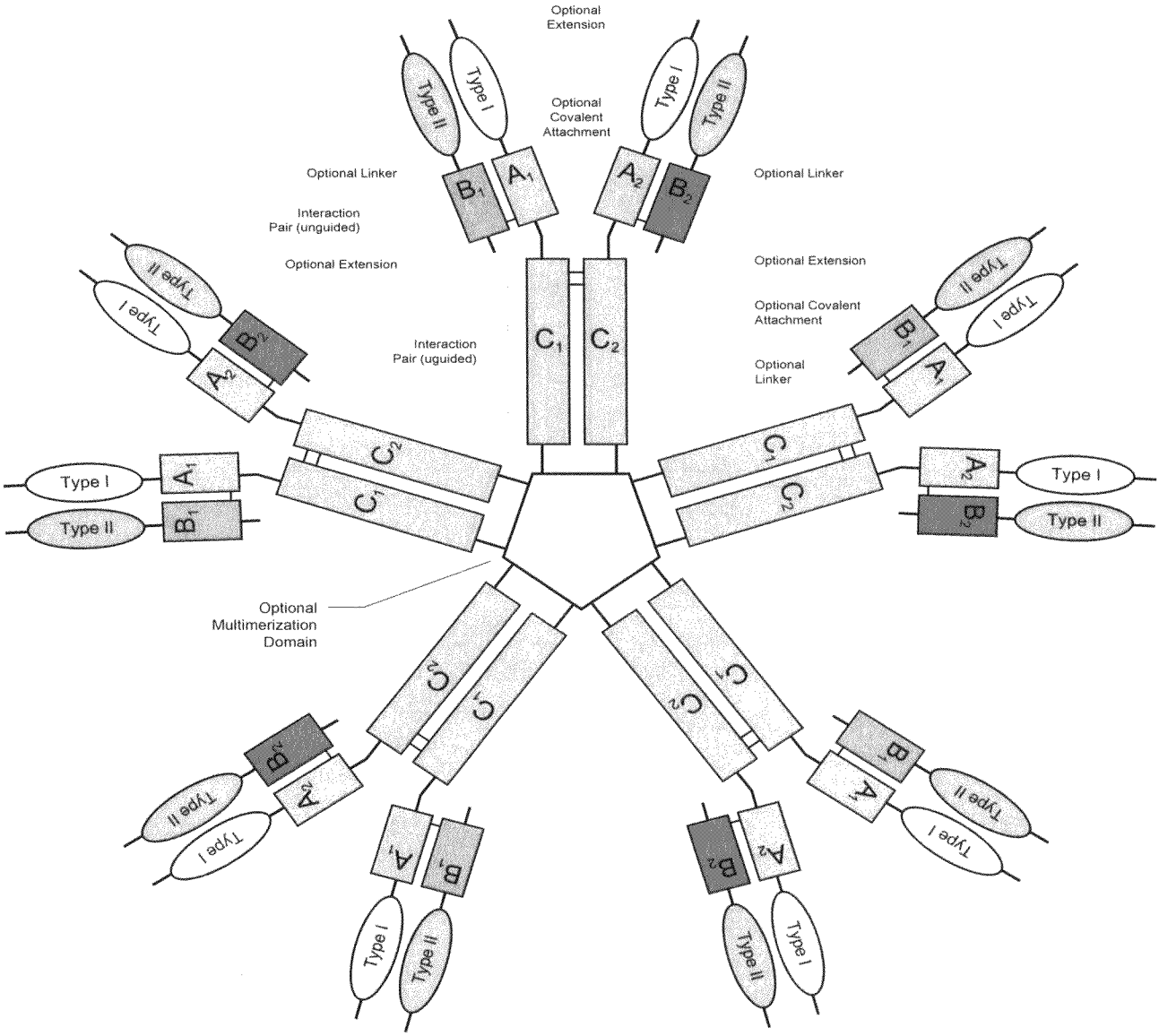

Optional Extension
Optional Covalent Attachment
Optional Linker
Interaction Pair (unguided)
Optional Extension
Interaction Pair (uguided)
Optional Linker
Optional Extension
Optional Covalent Attachment
Optional Linker
Type I
Type II
Optional Multimerization Domain

FIGURE 16D

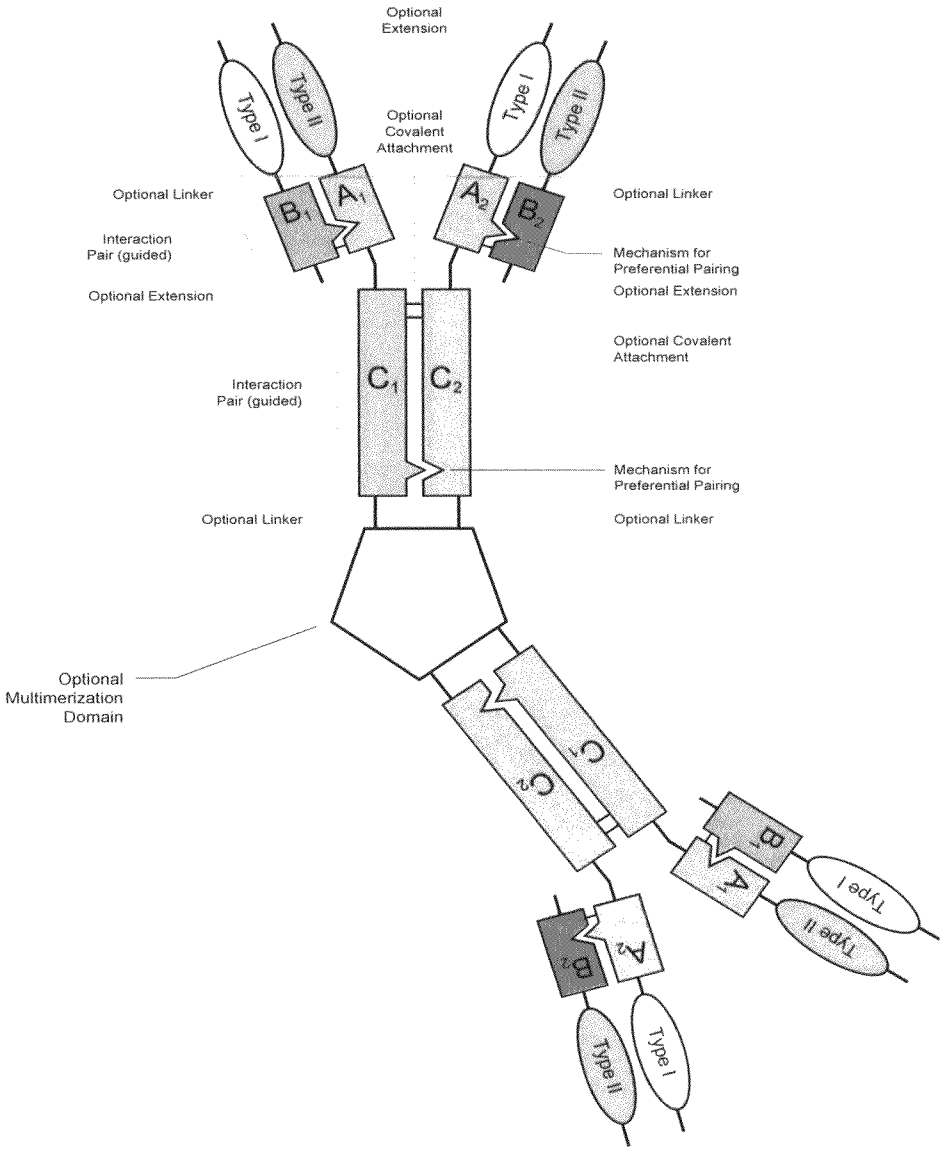

Optional Extension
Type I
Type II
Optional Covalent Attachment
Optional Linker
Interaction Pair (guided)
Optional Extension
Mechanism for Preferential Pairing
Optional Linker
Optional Extension
Optional Covalent Attachment
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Linker
Optional Linker
Optional Multimerization Domain

FIGURE 16E

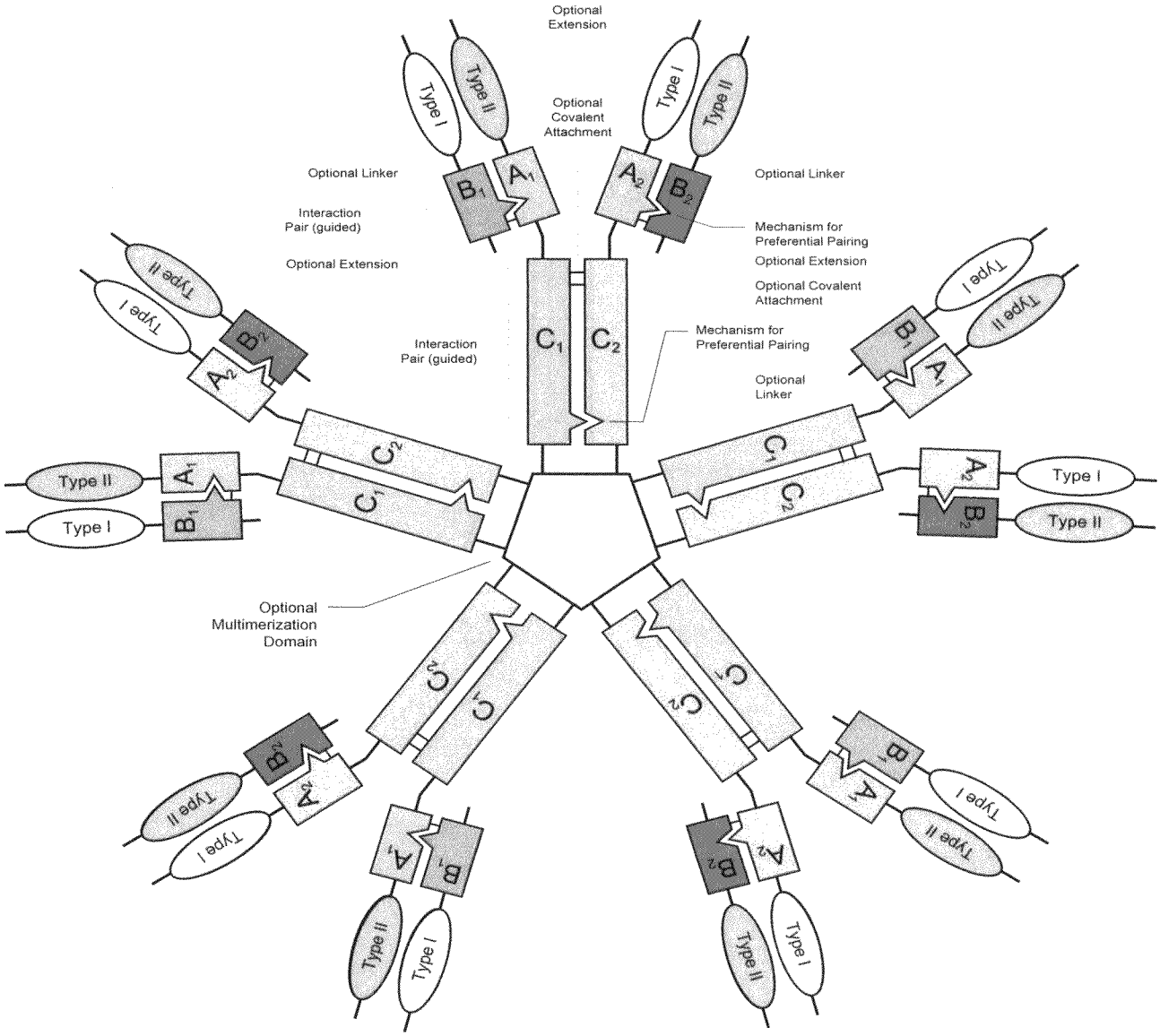
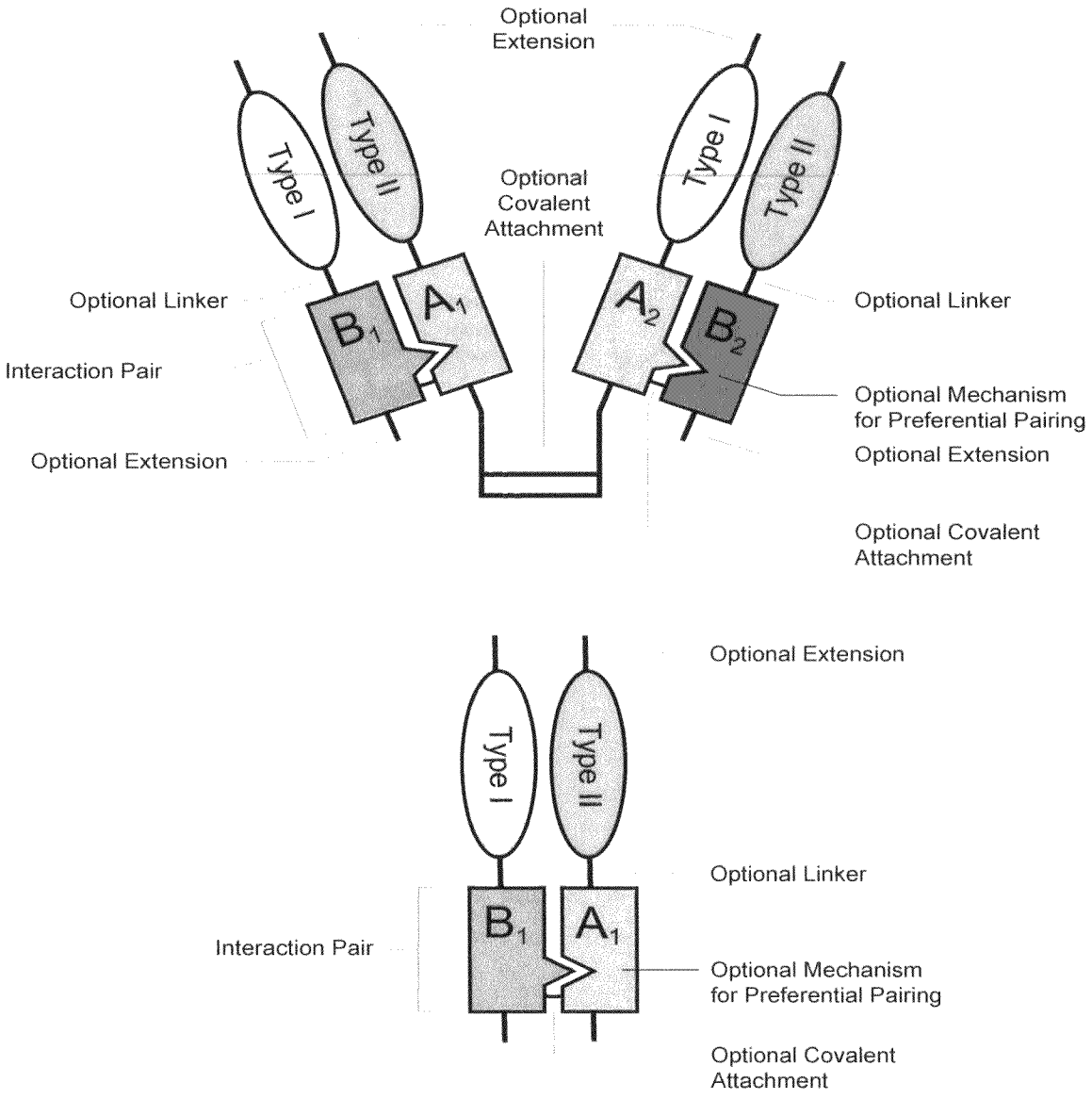

Optional Extension
Optional Covalent Attachment
Optional Linker
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Extension
Optional Covalent Attachment
Optional Linker
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Multimerization Domain
Type I
Type II

FIGURE 16F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Type I
Type II
Optional Covalent Attachment
Type I
Type II
Optional Linker
Interaction Pair
Optional Extension
B_1
A_1
A_2
B_2
Optional Linker
Optional Mechanism for Preferential Pairing
Optional Extension
Optional Covalent Attachment
Optional Extension
Type I
Type II
Optional Linker
Interaction Pair
B_1
A_1
Optional Mechanism for Preferential Pairing
Optional Covalent Attachment

FIGURE 16G

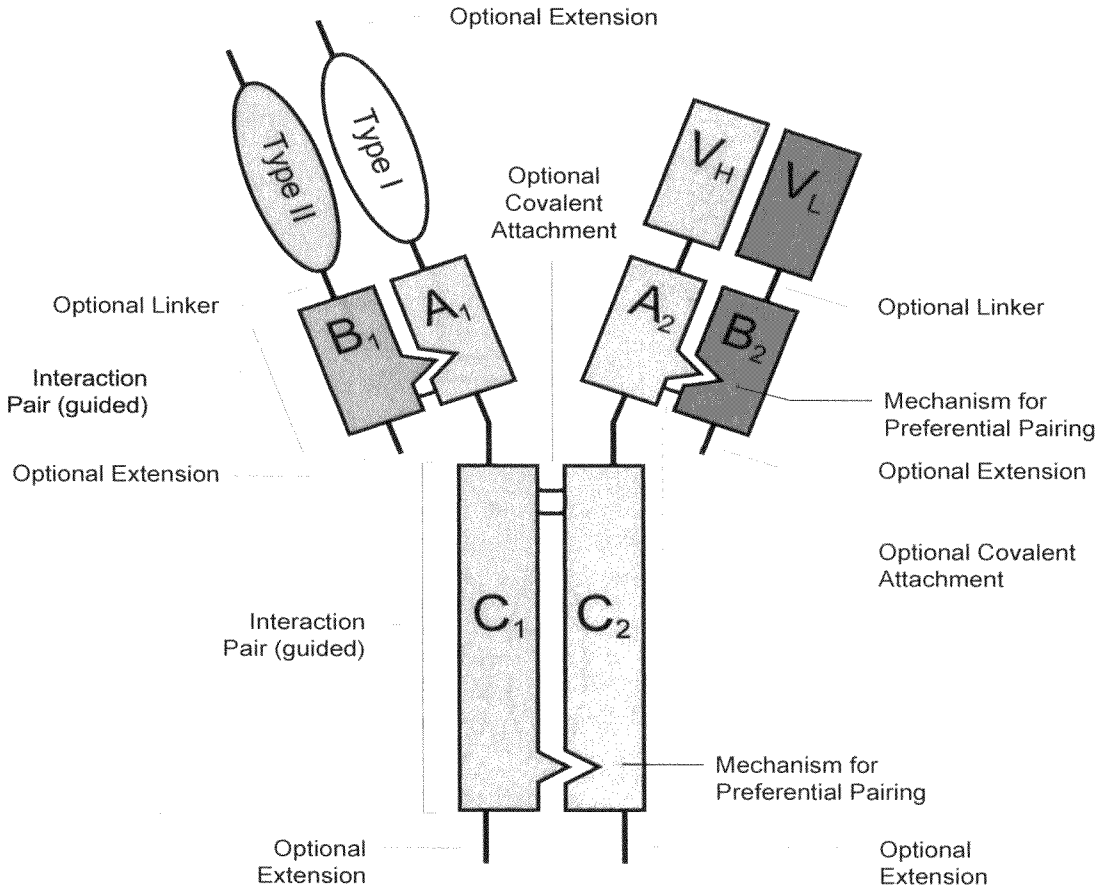
Optional Extension
Type II
Type I
Optional Covalent Attachment
V_H
V_L
Optional Linker
B_1
A_1
A_2
B_2
Optional Linker
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Extension
Optional Extension
Optional Covalent Attachment
Interaction Pair (guided)
C_1
C_2
Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 17A

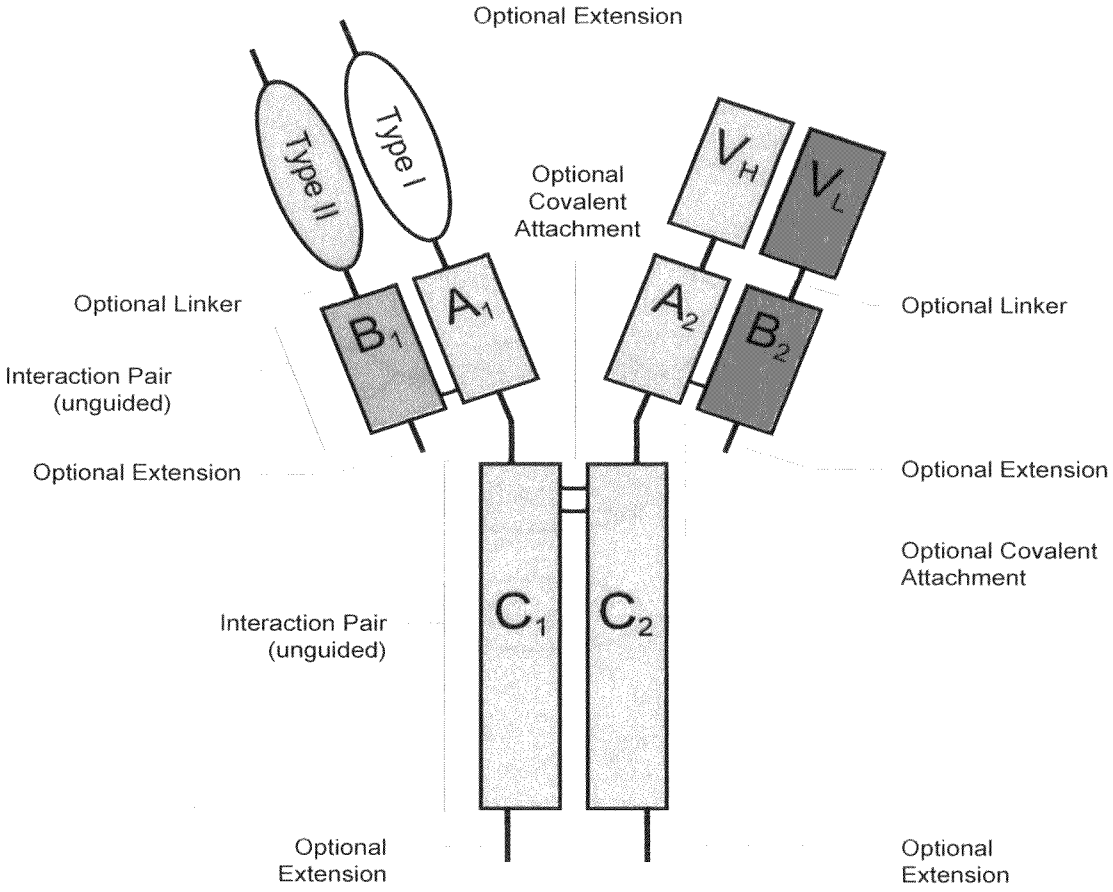
Optional Extension
Type II
Type I
Optional Covalent Attachment
V_H
V_L
Optional Linker
B_1
A_1
A_2
B_2
Optional Linker
Interaction Pair (unguided)
Optional Extension
Optional Extension
Optional Covalent Attachment
Interaction Pair (unguided)
C_1
C_2
Optional Extension
Optional Extension

FIGURE 17B

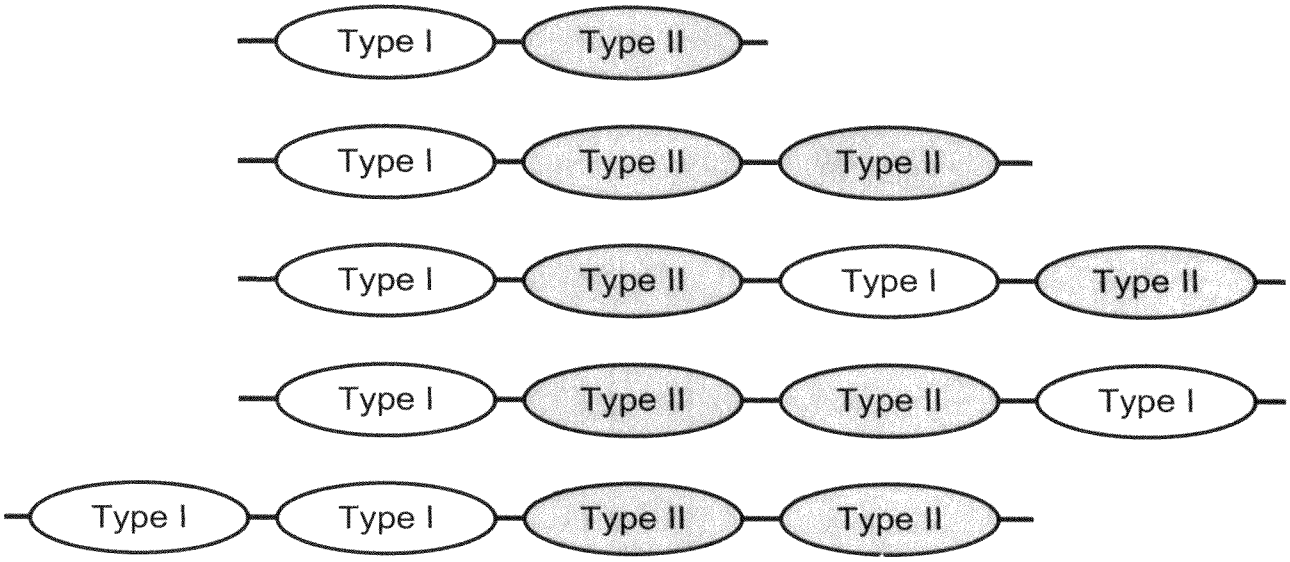
Type I
Type II
Type I
Type II
Type II
Type I
Type II
Type I
Type II
Type I
Type I
Type II
Type II
Type I
Type I
Type II
Type II

FIGURE 18

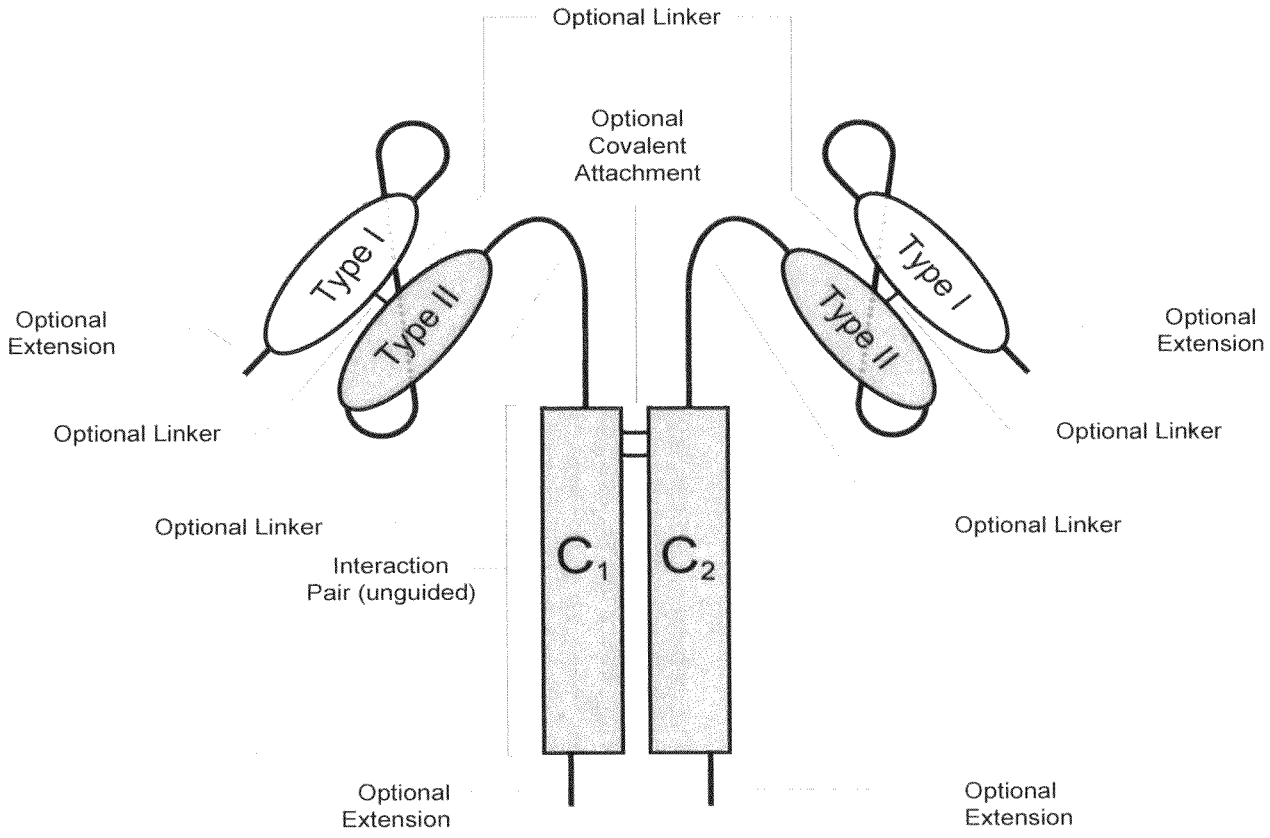
Optional Linker
Optional Covalent Attachment
Type I
Type II
Type I
Type II
Optional Extension
Optional Extension
Optional Linker
Optional Linker
Optional Linker
Optional Linker
Interaction Pair (unguided)
C1
C2
Optional Extension
Optional Extension

FIGURE 19A

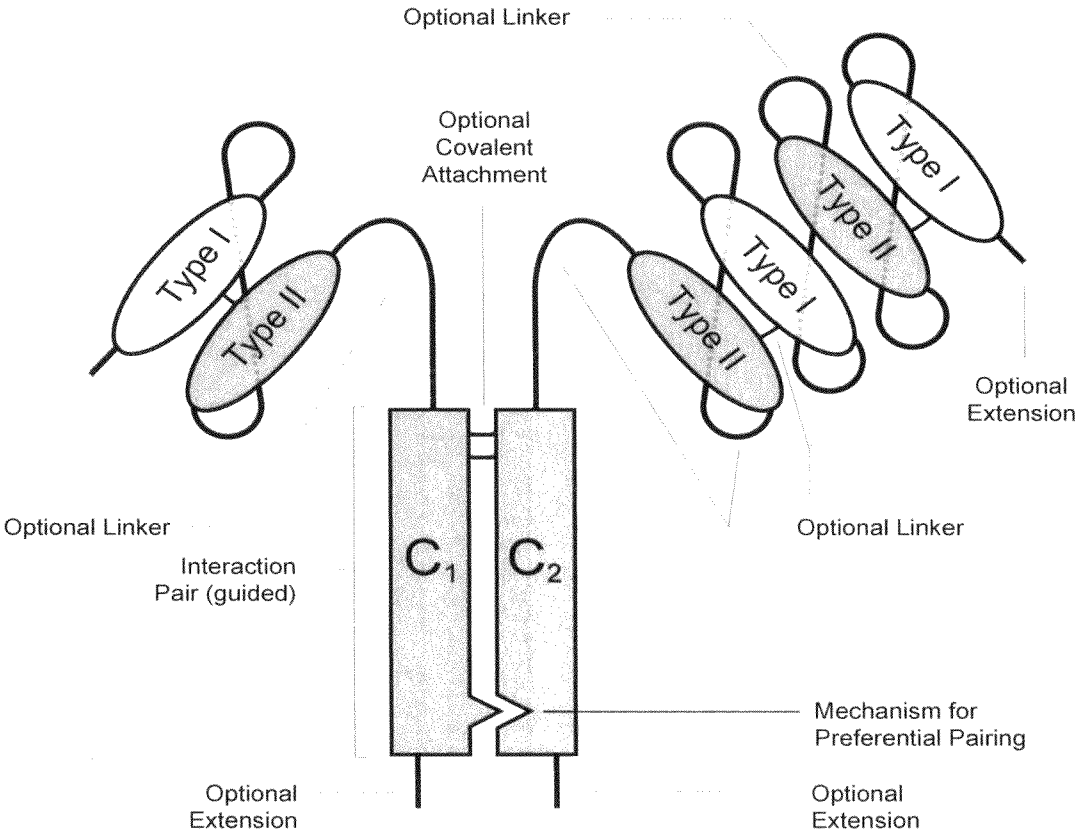
Optional Linker
Optional Covalent Attachment
Type I
Type II
Type I
Type II
Type I
Type II
Optional Extension
Optional Linker
Optional Linker
Interaction Pair (guided)
C1
C2
Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 19B

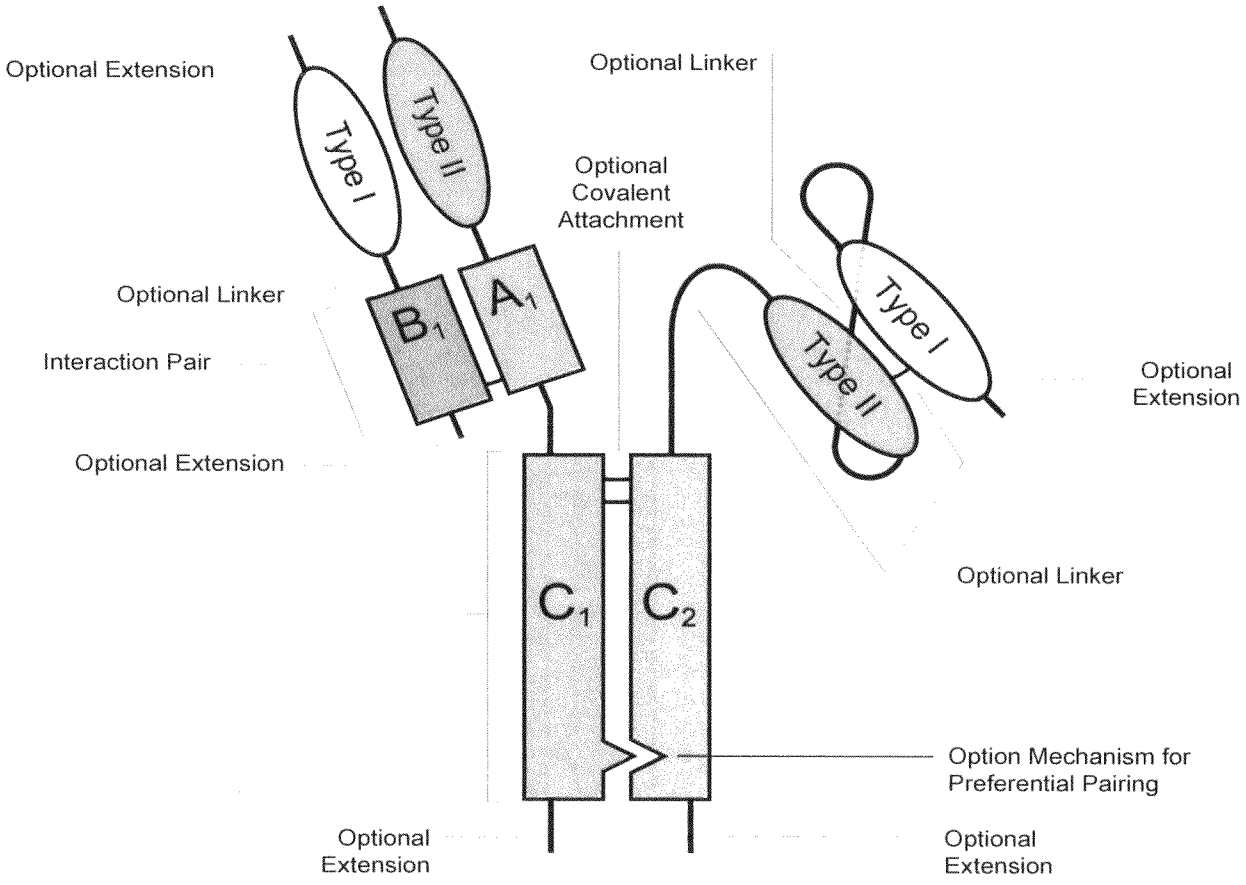
Optional Extension
Type I
Type II
Optional Linker
Optional Covalent Attachment
Optional Linker
Interaction Pair
B1
A1
Type I
Type II
Optional Extension
Optional Extension
Optional Linker
C1
C2
Option Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 19C

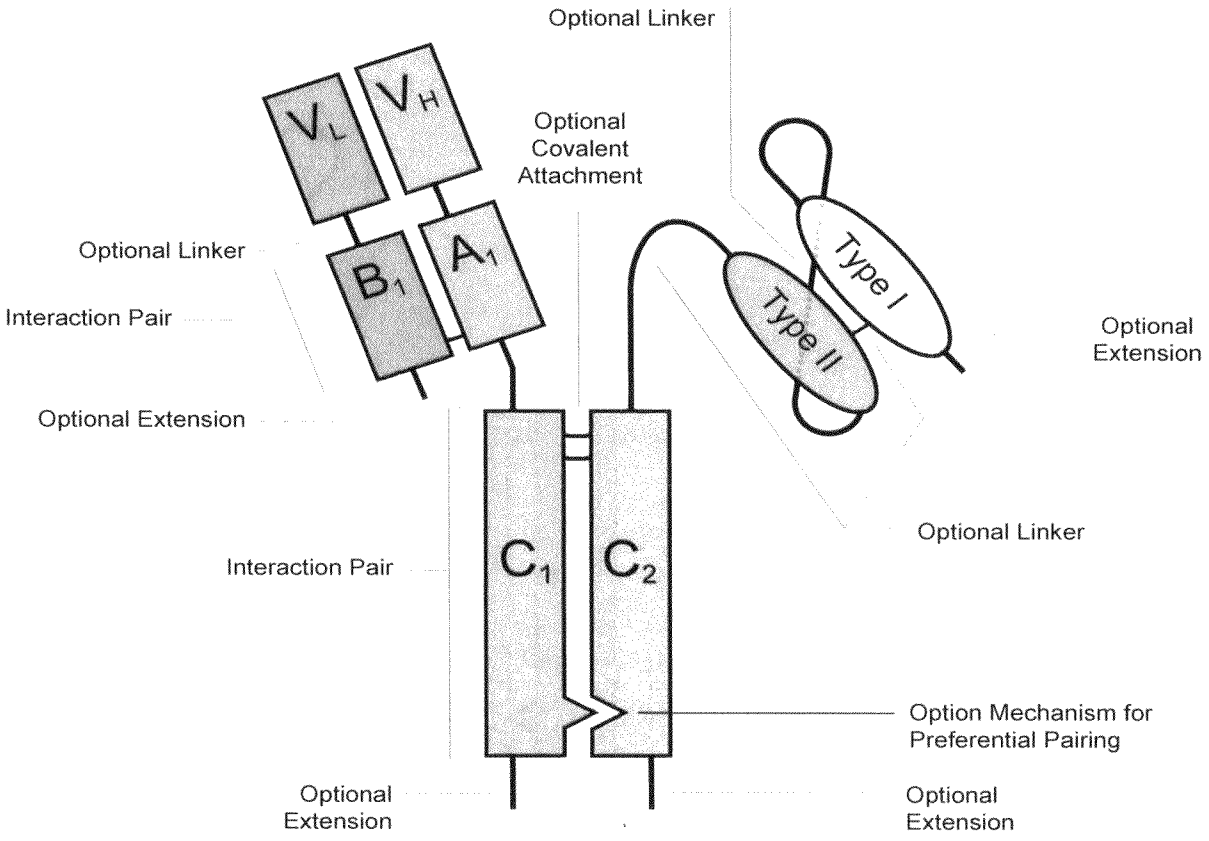
Optional Linker
V_L
V_H
Optional Covalent Attachment
Optional Linker
B_1
A_1
Interaction Pair
Type I
Type II
Optional Extension
Optional Extension
Optional Linker
Interaction Pair
C_1
C_2
Option Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 19D

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A61P 19/00 (2006.01) *C07K 14/71* (2006.01)
A61P 21/00 (2006.01) *C07K 19/00* (2006.01)

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6 April 2016 (06.04.2016)

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62/143,579 6 April 2015 (06.04.2015) US

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

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
8 December 2016

WO 2016/164089 A3

(54) Title: TGF-BETA SUPERFAMILY TYPE I AND TYPE II RECEPTOR HETEROMULTIMERS AND USES THEREOF

(57) Abstract: In certain aspects, the disclosure provides soluble heteromeric polypeptide complexes comprising an extracellular domain of a type I serine/threonine kinase receptor of the TGF-beta family and an extracellular domain of a type II serine/threonine kinase receptor of the TGF-beta family. In some embodiments, the disclosure provides soluble polypeptide complexes comprising an extracellular domain of a type II receptor selected from: ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII. In some embodiments, the disclosure provides soluble polypeptide complexes comprising an extracellular domain of a type I receptor selected from: ALK1, ALK.2, ALK3, ALK4, ALK5, ALK.6, and ALK7. Optionally the soluble complex is a heterodimer. In certain aspects, such soluble polypeptide complexes may be used to regulate (promote or inhibit) growth of tissues or cells including, for example, muscle, bone, cartilage, fat, neural tissue, tumors, cancerous cells, and/or cells of hematopoietic lineages, including red blood cells. In certain aspects, such soluble polypeptide complexes are can be used to improve muscle formation, bone formation, hematopoiesis, metabolic parameters, and disorders associated with these tissues, cellular networks, and endocrine systems.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/000033

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/000033

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/000033

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/18 (2006.01) ***A61K 39/395 (2006.01)*** ***A61P 19/00 (2006.01)*** ***A61P 21/00 (2006.01)*** ***A61P 9/00 (2006.01)*** ***A61P 35/00 (2006.01)*** ***C07K 14/71 (2006.01)*** ***C07K 19/00 (2006.01)***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIDS, Medline, Biosis & keywords: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, ActRIIB, Fc, multimer and like terms

GenomeQuest and SEQ ID Nos: 410 with 414, 422, 408, 404, 424, 426 or 406; 402 with 414, 422, 408, 404, 424, 426 or 406; 120 with 126, 138, 117, 106, 141, 144 or 114; 102 with 126, 138, 117, 106, 141, 144 or 114. All at 100% identity

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 November 2016	08 November 2016
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Nathalie Tochon-Danguy AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833101

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2016/000033**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CASTONGUAY R. et al.,"Soluble endoglin specifically binds bone morphogenetic proteins 9 and 10 via its orphan domain, inhibits blood vessel formation, and suppress tumor growth", THE JOURNAL OF BIOLOGICAL CHEMISTRY, 2011, vol.286, no. 34, pages 30034-30046 Materials, last paragraph of first column page 30035	1-15, 37-62, 69-86, 103-234, 497
A	WO 2009/134428 A2 (ACCELERON PHARMA, INC. et al.) 05 November 2009 abstract, Figures, Examples	1-15, 37-62, 69-86, 103-234, 497
A	WO 2008/076437 A2 (ACCELERON PHARMA, INC.) 26 June 2008 abstract, Figures, Examples	1-15, 37-62, 69-86, 103-234, 497
A	WO 2010/114860 A1 (ACCELERON PHARMA, INC.) 07 October 2010 abstract, Figures, Examples	1-15, 37-62, 69-86, 103-234, 497
A	WO 2005/084699 A1 (ACCELERON PHARMA, INC.) 15 September 2005 abstract, Figures, Examples	1-15, 37-62, 69-86, 103-234, 497

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2016/000033

Supplemental Box

Continuation of: **Box III**

The present set of 497 claims define a multitude of recombinant TGFβ heteromultimer complexes, pharmaceutical compositions comprising the complexes and methods of treating particular disorders and conditions by administering effective amounts of the complexes.

The 497 claims are characterised by multiple inventions between independent claims and multiple inventions within independent and dependent claims.

Accordingly, this ISA considers that the presentation of multiple inventions and numerous alternative embodiments within independent and dependent claims makes it difficult to conduct a meaningful novelty and inventive step search across their full scope, even if the claims are grouped into separate inventions.

Also, PCT Rule 33.3(b), indicates that for reasons of economy, certain restrictions of the international search may be necessary (see also PCT International Search and Preliminary Examination Guidelines at pargraph15.24).

It is also considered that some of the claims contravene Article 5 because the claimed subject matter is not commensurate with the description. Please see Box VIII of the written Opinion for further comment on this matter.

For the reasons outlined above, this ISA does not consider it reasonable or economical to identify the exact number of separate claimed inventions.

By way of example only, independent claim 1, is drawn to multiple embodiments of heteromultimer complexes comprising a TGFβ Type I receptor and a TGFβ Type II receptor.

In contrast:

- independent claim 495 defines heteromultimers of the Type II:Type II receptors;
- independent claim 496 (miss-numbered 460) defines Type I:Type I receptors;
- and independent claim 497 (miss-numbered 461) defines Type I:Type II receptors;

And claims 495, 496 and 497 all refer to numerous embodiments of each type of receptor as laid out in the tables at pages 333-336, 358-363 and 364-373.

Although claims 495 to 497 are all drawn to TGFβ heteromultimer complexes, the three separate categories of claimed complexes do not have a common structural or sequence feature that could be considered unifying between all the three groups of claimed complexes.

With respect to claim 1, there is no unity of invention between claim 1 and claims 495 or 496 at least.

This Search/Opinion is based on claims 1-15, 37-62, 69-86, 103-234 and 497 when limited to TGFβ Type I receptor polypeptides (ALK1, ALK2, ALK3, ALK4, ALK5, ALK6 or ALK 7) in a complex with (ActRIIa or ActRIIB) TGFβ Type II polypeptide receptors.

Furthermore, with respect to ActRIIB combined with TGFβ Type I polypeptides the search was further limited to complexes defined by combinations of SEQ ID Nos: (102:126); (402:414); (102:138); (402: 422); (102:117); (402;408); (102:106); (402:404);(102:141); (402:424); (102:144); (402:426); (102:114);(402:406) and all only with respect to 100% identity of polypeptide sequence.

Similarly, with respect to ActRIIA combined with TGFβ Type I polypeptides the search was further limited to complexes defined by combinations of SEQ ID Nos: (120:126); (410:414); (120:138); (410:422); (120:117); (410:408); (120:106); (410:404); (120:141); (410:424); (120:144); (410:426); (120:114); (410:406); and all only with respect to 100% identity of polypeptide sequence.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/000033**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2009/134428 A2	05 November 2009	WO 2009134428 A2	05 Nov 2009
		AU 2007317926 A1	15 May 2008
		AU 2009241755 A1	05 Nov 2009
		AU 2009241755 B2	01 Oct 2015
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		BR PI0717964 A2	17 Dec 2013
		BR PI0911853 A2	14 Jun 2016
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		JP 2011520793 A	21 Jul 2011
		JP 5719766 B2	20 May 2015
		JP 2010508816 A	25 Mar 2010
		JP 2013199496 A	03 Oct 2013
		JP 2015042680 A	05 Mar 2015
		JP 2015143269 A	06 Aug 2015
		KR 20090085662 A	07 Aug 2009
		KR 101581961 B1	04 Jan 2016
		KR 20110031908 A	29 Mar 2011
		KR 20150008889 A	23 Jan 2015
		MX 2009004718 A	19 Jun 2009
		MX 2010011998 A	11 Apr 2011
		RU 2009120695 A	10 Dec 2010
		RU 2013134324 A	27 Jan 2015
		TW 200950806 A	16 Dec 2009
		TW 201609138 A	16 Mar 2016

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/000033**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2009311272 A1	17 Dec 2009
		US 8158584 B2	17 Apr 2012
		US 2008175844 A1	24 Jul 2008
		US 8455428 B2	04 Jun 2013
		US 2009304691 A1	10 Dec 2009
		US 8642031 B2	04 Feb 2014
		US 2014227254 A1	14 Aug 2014
		US 9452197 B2	27 Sep 2016
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		US 2013040896 A1	14 Feb 2013
		US 2013344067 A1	26 Dec 2013
		WO 2008057461 A2	15 May 2008
		WO 2009139891 A2	19 Nov 2009

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/000033**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2008/076437 A2	26 June 2008	WO 2008076437 A2	26 Jun 2008
		AU 2007334333 A1	26 Jun 2008
		AU 2007334333 A2	12 May 2011
		AU 2007334333 B2	30 May 2013
		AU 2009262968 A1	30 Dec 2009
		AU 2016203098 A1	02 Jun 2016
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		CA 2672758 A1	26 Jun 2008
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		CN 102131515 A	20 Jul 2011
		CN 104524548 A	22 Apr 2015
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		CO 6230995 A2	20 Dec 2010
		CR 10889 A	01 Sep 2009
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		EP 2124999 A2	02 Dec 2009
		EP 2124999 B1	03 Oct 2012
		EP 2303917 A2	06 Apr 2011
		EP 2318028 A2	11 May 2011
		EP 2446896 A1	02 May 2012
		EP 2468290 A1	27 Jun 2012
		EP 2468290 B1	01 Apr 2015
		EP 2468291 A1	27 Jun 2012
		HK 1137368 A1	01 Mar 2013
		HK 1209624 A1	08 Apr 2016
		IL 199451 A	31 Aug 2015
		JP 2010513506 A	30 Apr 2010
		JP 5415279 B2	12 Feb 2014

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/000033**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/000033**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
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INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/000033**

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Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

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权利要求书49页 说明书250页 附图24页

(54)发明名称

TGF-β超家族I型和II型受体异多聚体及其用途

(57)摘要

在某些方面,本公开内容提供包含TGF-β家族的I型丝氨酸/苏氨酸激酶受体的胞外域和TGF-β家族的II型丝氨酸/苏氨酸激酶受体的胞外域的可溶性异聚体多肽复合体。在一些实施方案中,本公开内容提供包含选自以下II型受体的胞外域的可溶性多肽复合体:ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII。在一些实施方案中,本公开内容提供包含选自以下I型受体的胞外域的可溶性多肽复合体:ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7。任选可溶性复合体是异二聚体。在某些方面,这类可溶性多肽复合体可用于调节(促进或抑制)组织或细胞(包括例如肌肉、骨、软骨、脂肪、神经组织、肿瘤、癌细胞和/或造血谱系细胞,包括红细胞)的生长。在某些方面,这类可溶性多肽复合体可用来改善肌肉形成、骨形成、血细胞生成、代谢参数和与这些组织、细胞网和内分泌系统有关的病症。

CN 107847561 A

1.一种重组异多聚体复合体,其包含TGFβ超家族I型受体多肽,任选TGFβ超家族I型受体的胞外域,和TGFβ超家族II型受体多肽,任选TGFβ超家族II型受体的胞外域,其中所述TGFβ超家族I型受体多肽选自:ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7,其中所述TGFβ超家族II型受体多肽选自:ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII。

2.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK1多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIA多肽。

3.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK2多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIA多肽。

4.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK3多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIA多肽。

5.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK4多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIA多肽。

6.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK5多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIA多肽。

7.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK6多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIA多肽。

8.权利要求1的异多聚体复合体,其中所述TGFβ超家族T型受体多肽是ALK7多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIA多肽。

9.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK1多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIB多肽。

10.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK2多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIB多肽。

11.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK3多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIB多肽。

12.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK4多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIB多肽。

13.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK5多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIB多肽。

14.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK6多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIB多肽。

15.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK7多肽,且其中所述TGFβ超家族II型受体多肽是ActRIIB多肽。

16.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK1多肽,且其中所述TGFβ超家族II型受体多肽是TGFBRII多肽。

17.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK2多肽,且其中所述TGFβ超家族II型受体多肽是TGFBRII多肽。

18.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK3多肽,且其中所述TGFβ超家族II型受体多肽是TGFBRII多肽。

19.权利要求1的异多聚体复合体,其中所述TGFβ超家族I型受体多肽是ALK4多肽,且其

中所述TGFβ超家族II型受体多肽是TGFBRII多肽。

20.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK5多肽，且其中所述TGFβ超家族II型受体多肽是TGFBRII多肽。

21.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK6多肽，且其中所述TGFβ超家族II型受体多肽是TGFBRII多肽。

22.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK7多肽，且其中所述TGFβ超家族II型受体多肽是TGFBRII多肽。

23.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK1多肽，且其中所述TGFβ超家族II型受体多肽是BMPRII多肽。

24.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK2多肽，且其中所述TGFβ超家族II型受体多肽是BMPRII多肽。

25.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK3多肽，且其中所述TGFβ超家族II型受体多肽是BMPRII多肽。

26.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK4多肽，且其中所述TGFβ超家族II型受体多肽是BMPRII多肽。

27.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK5多肽，且其中所述TGFβ超家族II型受体多肽是BMPRII多肽。

28.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK6多肽，且其中所述TGFβ超家族II型受体多肽是BMPRII多肽。

29.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK7多肽，且其中所述TGFβ超家族II型受体多肽是BMPRII多肽。

30.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK1多肽，且其中所述TGFβ超家族II型受体多肽是MISRII多肽。

31.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK2多肽，且其中所述TGFβ超家族II型受体多肽是MISRII多肽。

32.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK3多肽，且其中所述TGFβ超家族II型受体多肽是MISRII多肽。

33.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK4多肽，且其中所述TGFβ超家族II型受体多肽是MISRII多肽。

34.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK5多肽，且其中所述TGFβ超家族II型受体多肽是MISRII多肽。

35.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK6多肽，且其中所述TGFβ超家族II型受体多肽是MISRII多肽。

36.权利要求1的异多聚体复合体，其中所述TGFβ超家族I型受体多肽是ALK7多肽，且其中所述TGFβ超家族II型受体多肽是MISRII多肽。

37.权利要求2、9、16、23或30中任一项的异多聚体复合体，其中所述ALK1多肽包含与SEQ ID NO:15的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

38.权利要求2、9、16、23或30中任一项的异多聚体复合体，其中所述ALK1多肽包含SEQ

ID NO:15的氨基酸序列、由其组成或基本由其组成。

39.权利要求3、10、17、24或31中任一项的异多聚体复合体，其中所述ALK2多肽包含与SEQ ID NO:19的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

40.权利要求3、10、17、24或31中任一项的异多聚体复合体，其中所述ALK2多肽包含SEQ ID NO:19的氨基酸序列、由其组成或基本由其组成。

41.权利要求4、11、18、25或32中任一项的异多聚体复合体，其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸31-140有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

42.权利要求4、11、18、25或32中任一项的异多聚体复合体，其中所述ALK3多肽包含SEQ ID NO:22的氨基酸31-140、由其组成或基本由其组成。

43.权利要求4、11、18、25或32中任一项的异多聚体复合体，其中所述ALK3多肽包含与SEQ ID NO:23的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

44.权利要求4、11、18、25或32中任一项的异多聚体复合体，其中所述ALK3多肽包含SEQ ID NO:23的氨基酸序列、由其组成或基本由其组成。

45.权利要求5、12、19、26或33中任一项的异多聚体复合体，其中所述ALK4多肽包含与选自SEQ ID NO:27、84、104、106、403和404的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

46.权利要求5、12、19、26或33中任一项的异多聚体复合体，其中所述ALK4多肽包含选自SEQ ID NO:27、84、104、106、403和404的氨基酸序列、由其组成或基本由其组成。

47.权利要求6、13、20、27或34中任一项的异多聚体复合体，其中所述ALK5多肽包含与SEQ ID NO:31或88的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

48.权利要求6、13、20、27或34中任一项的异多聚体复合体，其中所述ALK5多肽包含SEQ ID NO:31或88的氨基酸序列、由其组成或基本由其组成。

49.权利要求7、14、21、28或35中任一项的异多聚体复合体，其中所述ALK6多肽包含与SEQ ID NO:35或92的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

50.权利要求7、14、21、28或35中任一项的异多聚体复合体，其中所述ALK6多肽包含SEQ ID NO:35或92的氨基酸序列、由其组成或基本由其组成。

51.权利要求8、15、22、29或36中任一项的异多聚体复合体，其中ALK7多肽包含与选自SEQ ID NO:39、112、114、302、306、310、313、405和406的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

52.权利要求8、15、22、29或36中任一项的异多聚体复合体，其中所述ALK7多肽包含选自SEQ ID NO:39、112、114、302、306、310、313、405和406的氨基酸序列、由其组成或基本由其组成。

53.权利要求2-8中任一项的异多聚体复合体，其中所述ActRIIA多肽包含与选自SEQ ID NO:10或11的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基

酸序列。

54.权利要求2-8中任一项的异多聚体复合体，其中所述ActRIIA多肽包含选自SEQ ID NO:10或11的氨基酸序列、由其组成或基本由其组成。

55.权利要求9-15中任一项的异多聚体复合体，其中所述ActRIIB多肽包含与选自SEQ ID NO:2、3、5、6、100、102、401和402的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

56.权利要求9-15中任一项的异多聚体复合体，其中所述ActRIIB多肽包含与选自SEQ ID NO:2、3、5或6的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列，且其中对应于SEQ ID NO:1的L79的位置是酸性氨基酸(即D或E氨基酸残基)。

57.权利要求9-15中任一项的异多聚体复合体，其中所述ActRIIB多肽包含选自SEQ ID NO:2、3、5、6、100、102、401和402的氨基酸序列、由其组成或基本由其组成。

58.权利要求9-15中任一项的异多聚体复合体，其中所述ActRIIB多肽包含选自SEQ ID NO:2、3、5或6的氨基酸序列、由其组成或基本由其组成，且其中对应于SEQ ID NO:1的L79的位置是酸性氨基酸(即D或E氨基酸残基)。

59.权利要求9-15中任一项的异多聚体复合体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列。

60.权利要求9-15中任一项的异多聚体复合体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列，且其中对应于SEQ ID NO:1的L79的位置是酸性氨基酸(即D或E氨基酸残基)。

61.权利要求9-15中任一项的异多聚体复合体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-131有至少70％、75％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列。

62.权利要求9-15中任一项的异多聚体复合体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-131有至少70％、75％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列，且其中对应于SEQ ID NO:1的L79的位置是酸性氨基酸(即D或E氨基酸残基)。

63.权利要求16-22中任一项的异多聚体复合体，其中所述TGFBRII多肽包含与SEQ ID NO:43、68、129或416的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

64.权利要求16-22中任一项的异多聚体复合体，其中所述TGFBRII多肽包含SEQ ID NO:43、68、129或416的氨基酸序列、由其组成或基本由其组成。

65.权利要求23-29中任一项的异多聚体复合体，其中所述BMPRII多肽包含与SEQ ID NO:47、72、123或412的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

66.权利要求23-29中任一项的异多聚体复合体，其中所述BMPRII多肽包含SEQ ID NO:47、72、123或412的氨基酸序列、由其组成或基本由其组成。

67.权利要求30-36中任一项的异多聚体复合体，其中所述MISRII多肽包含与SEQ ID

NO:51、76或80的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

68.权利要求30-36中任一项的异多聚体复合体,其中所述MISRII多肽包含SEQ ID NO:51、76或80的氨基酸序列、由其组成或基本由其组成。

69.权利要求1-68中任一项的异多聚体复合体,其中所述复合体是异二聚体。

70.权利要求1-69中任一项的异多聚体复合体,其中所述TGFβ超家族I型受体多肽的胞外域是另包含至少一个异源部分,任选相互作用对的第一或第二成员的融合蛋白。

71.权利要求1-70中任一项的异多聚体复合体,其中所述TGFβ超家族II型受体多肽的胞外域是另包含至少一个异源部分,任选相互作用对的第一或第二成员的融合蛋白。

72.权利要求70或71的异多聚体复合体,其中所述异源部分包含来自IgG重链的恒定区。

73.权利要求72的异多聚体复合体,其中所述来自IgG重链的恒定区是免疫球蛋白Fc结构域。

74.权利要求72或73的异多聚体复合体,其中所述异源部分包含与选自SEQ ID NO:200-212中任一个的序列有至少70％、80％、85％、90％、95％、97％、98％或99％同一性的氨基酸序列。

75.权利要求73的异多聚体复合体,其中所述免疫球蛋白Fc结构域包含选自SEQ ID NO:200-212中任一个的氨基酸序列、由其组成或基本由其组成。

76.权利要求73-75中任一项的异多聚体复合体,其中所述免疫球蛋白Fc结构域包含促进异二聚体形成的一个或多个氨基酸突变。

77.权利要求73-76中任一项的异多聚体复合体,其中所述免疫球蛋白Fc结构域包含抑制同二聚体形成的一个或多个氨基酸突变。

78.权利要求1-77中任一项的异多聚体复合体,其中所述TGFβ超家族I型受体多肽的胞外域包含选自以下的一个或多个修饰的氨基酸残基:糖基化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化氨基酸、与脂质部分缀合的氨基酸和与有机衍化剂缀合的氨基酸。

79.权利要求1-78中任一项的异多聚体复合体,其中所述TGFβ超家族II型受体多肽的胞外域包含选自以下的一个或多个修饰的氨基酸残基:糖基化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化氨基酸、与脂质部分缀合的氨基酸和与有机衍化剂缀合的氨基酸。

80.权利要求78的异多聚体复合体,其中所述TGFβ超家族I型受体多肽被糖基化,并且具有可获自I型受体多肽在CHO细胞中表达的糖基化模式。

81.权利要求79的异多聚体复合体,其中所述TGFβ超家族1I型受体多肽被糖基化,并且具有可获自I型受体多肽在CHO细胞中表达的糖基化模式。

82.权利要求1-81中任一项的异多聚体复合体,其中所述异多聚体复合体具有以下特征的一项或多项:

i)以至少10^{-7}M的K_D与TGF-β超家族配体结合;和

ii)在细胞中抑制TGFβ超家族I型和/或II型受体介导的信号转导。

83.权利要求1-82中任一项的异多聚体复合体,其中所述异多聚体复合体与以下的一

种或多种结合:BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty。

84.权利要求1-83中任一项的异多聚体复合体,其中所述异多聚体复合体在基于细胞的测定法中抑制一种或多种TGF-β超家族配体的活性。

85.权利要求84的异多聚体复合体,其中所述TGF-β超家族配体选自:BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty。

86.一种药物制剂,其包含权利要求1-85中任一项的异多聚体复合体和药学上可接受的载体。

87.一种用于治疗患有与肌肉丢失或肌肉生长不足有关的病症的患者的方法,所述方法包括给予有需要的患者有效量的权利要求1-85中任一项的异多聚体复合体。

88.权利要求87的方法,其中所述患者患有肌肉萎缩。

89.权利要求87的方法,其中所述患者患有肌营养不良。

90.权利要求89的方法,其中所述肌营养不良是杜兴型肌营养不良。

91.权利要求90的方法,其中所述患者是青少年,治疗在杜兴型肌营养不良确诊日期的1-5年内开始。

92.权利要求89的方法,其中所述肌营养不良是面肩胛肱骨肌营养不良。

93.权利要求87的方法,其中所述患者患有肌萎缩性侧索硬化。

94.权利要求93的方法,其中所述患者在诊断为肌萎缩性侧索硬化之后接受治疗。

95.权利要求87的方法,其中所述病症是与癌症或癌症疗法有关的恶病质。

96.一种用于治疗患有与神经变性有关的病症的患者的方法,所述方法包括给予有需要的患者有效量的权利要求1-85中任一项的异多聚体复合体。

97.权利要求96的方法,其中所述病症是肌萎缩性侧索硬化。

98.一种用于在受试者中降低体脂肪含量或减缓体脂肪含量增加的速率的方法,所述方法包括给予有需要的受试者有效量的权利要求1-85中任一项的异多聚体复合体。

99.一种用于治疗与受试者的非期望的体重增加有关的病症的方法,所述方法包括给予有需要的受试者有效量的权利要求1-85中任一项的异多聚体复合体。

100.权利要求99的方法,其中所述病症选自:肥胖症、非胰岛素依赖型糖尿病(NIDDM)、心血管疾病、高血压、骨关节炎、中风、呼吸问题和胆囊疾病。

101.一种用于降低患者的胆固醇和/或甘油三酯的方法,所述方法包括给予有需要的受试者有效量的权利要求1-85中任一项的异多聚体复合体。

102.一种用于治疗肌肉衰减综合征的方法,所述方法包括给予有需要的患者有效量的权利要求1-85中任一项的异多聚体复合体。

103.一种包含ALK1多肽和ActRIIA多肽的重组异多聚体。

104.权利要求103的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸30-110有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

105.权利要求103的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸21-135有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

106.权利要求103的异多聚体，其中所述ActRIIA多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:9的氨基酸21-30的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29或30)，和

b)止于SEQ ID NO:9的氨基酸110-135的任一个(例如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或135)。

107.权利要求103-106中任一项的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

108.权利要求103-106的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

109.权利要求103-106的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸22-118有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

110.权利要求103-106的异多聚体，其中所述ALK1多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:14的氨基酸22-34的任一个(例如氨基酸残基22、23、24、25、26、27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:14的氨基酸95-118的任一个(例如氨基酸残基95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117和118)。

111.权利要求103-106的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

112.一种包含ALK2多肽和ActRIIA多肽的重组异多聚体。

113.权利要求112的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸30-110有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、

95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

114.权利要求112的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸21-135有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

115.权利要求112的异多聚体，其中所述ActRIIA多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:9的氨基酸21-30的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29或30)，和

b)止于SEQ ID NO:9的氨基酸110-135的任一个(例如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或135)。

116.权利要求112-115中任一项的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

117.权利要求112-115的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸35-99有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

118.权利要求112-115的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸21-123有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

119.权利要求112-115的异多聚体，其中所述ALK2多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:18的氨基酸21-35的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29、30、31、32、33、34和35)，和

b)止于SEQ ID NO:18的氨基酸99-123的任一个(例如氨基酸残基99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122和123)。

120.权利要求112-115的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

121.一种包含ALK3多肽和ActRIIA多肽的重组异多聚体。

122.权利要求121的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

123.权利要求121的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸21-

135有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

124.权利要求121的异多聚体，其中所述ActRIIA多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:9的氨基酸21-30的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29或30)，和

b)止于SEQ ID NO:9的氨基酸110-135的任一个(例如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或135)。

125.权利要求121-124中任一项的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

126.权利要求121-124的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸61-130有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

127.权利要求121-124的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸24-152有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

128.权利要求121-124的异多聚体，其中所述ALK3多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:22的氨基酸24-61的任一个(例如氨基酸残基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和61)，和

b)止于SEQ ID NO:22的氨基酸130-152的任一个(例如氨基酸残基130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151和152)。

129.权利要求121-124的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

130.一种包含ALK4多肽和ActRIIA多肽的重组异多聚体。

131.权利要求130的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸30-110有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

132.权利要求130的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸21-135有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、

95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

133.权利要求130的异多聚体，其中所述ActRIIA多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:9的氨基酸21-30的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29或30)，和

b)止于SEQ ID NO:9的氨基酸110-135的任一个(例如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或135)。

134.权利要求130-133中任一项的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

135.权利要求130-133的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸34-101有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

136.权利要求130-133的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸23-126有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

137.权利要求130-133的异多聚体，其中所述ALK4多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:26或83的氨基酸23-34的任一个(例如氨基酸残基23、24、25、26、27、28、29、30、31、32、33、34)，和

b)止于SEQ ID NO:26或83的氨基酸101-126的任一个(例如氨基酸残基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和126)。

138.权利要求130-133的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

139.一种包含ALK5多肽和ActRIIA多肽的重组异多聚体。

140.权利要求139的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

141.权利要求139的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸21-135有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

142.权利要求139的异多聚体，其中所述ActRIIA多肽包含与下列多肽有至少70%、

75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:9的氨基酸21-30的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29或30)，和

b)止于SEQ ID NO:9的氨基酸110-135的任一个(例如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或135)。

143.权利要求139-142中任一项的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

144.权利要求139-142的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸36-106有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

145.权利要求139-142的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸25-126有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

146.权利要求139-142的异多聚体，其中所述ALK5多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:30或87的氨基酸25-36的任一个(例如氨基酸残基25、26、27、28、29、30、31、32、33、34、35和36)，和

b)止于SEQ ID NO:30或87的氨基酸106-126的任一个(例如氨基酸残基106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

147.权利要求139-142的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

148.一种包含ALK6多肽和ActRIIA多肽的重组异多聚体。

149.权利要求148的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

150.权利要求148的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸21-135有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

151.权利要求148的异多聚体，其中所述ActRIIA多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:9的氨基酸21-30的任一个(例如氨基酸残基21、22、23、24、25、26、

27、28、29或30)，和

b)止于SEQ ID NO:9的氨基酸110-135的任一个(例如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或135)。

152.权利要求148-151中任一项的异多聚体，其中所述ActRIIA多肽包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

153.权利要求148-151的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸32-102有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

154.权利要求148-151的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸14-126有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

155.权利要求148-151的异多聚体，其中所述ALK6多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32)，和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

156.权利要求148-151的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

157.权利要求148-151的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

158.权利要求148-151的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸26-156有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

159.权利要求148-151的异多聚体，其中所述ALK6多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32)，和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、

125和126)。

160.一种包含ALK7多肽和ActRIIA多肽的重组异多聚体。

161.权利要求160的异多聚体,其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

162.权利要求160的异多聚体,其中所述ActRIIA多肽包含与SEQ ID NO:9的氨基酸21-135有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

163.权利要求160的异多聚体,其中所述ActRIIA多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:9的氨基酸21-30的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29或30),和

b)止于SEQ ID NO:9的氨基酸110-135的任一个(例如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或135)。

164.权利要求160-163中任一项的异多聚体,其中所述ActRIIA多肽包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

165.权利要求160-163的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸28-92有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

166.权利要求160-163的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸21-133有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

167.权利要求160-163的异多聚体,其中所述ALK7多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:38、305或309的氨基酸21-28的任一个(例如氨基酸残基21、22、23、24、25、26、27和28),和

b)止于SEQ ID NO:38、305或309的氨基酸92-133的任一个(例如氨基酸残基92、93、94、95、96、97、98、99、100、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、126、127、128、129、130、131、132和133)。

168.权利要求160-163的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

169.一种包含ALK1多肽和ActRIIB多肽的重组异多聚体。

170.权利要求169的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

171.权利要求169的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-131有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

172.权利要求169的异多聚体，其中所述ActRIIB多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:1的氨基酸20-29的任一个(例如氨基酸残基20、21、22、23、24、25、26、27、28或29)，和

b)止于SEQ ID NO:1的氨基酸109-134的任一个(例如氨基酸残基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或134)。

173.权利要求172中任一项的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

174.权利要求169-172的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

175.权利要求169-172的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸22-118有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

176.权利要求169-172的异多聚体，其中所述ALK1多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:14的氨基酸22-34的任一个(例如氨基酸残基22、23、24、25、26、27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:14的氨基酸95-118的任一个(例如氨基酸残基95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117和118)。

177.权利要求169-172的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

178.一种包含ALK2多肽和ActRIIB多肽的重组异多聚体。

179.权利要求178的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-

109有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

180.权利要求178的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-131有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

181.权利要求178的异多聚体，其中所述ActRIIB多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:1的氨基酸20-29的任一个(例如氨基酸残基20、21、22、23、24、25、26、27、28或29)，和

b)止于SEQ ID NO:1的氨基酸109-134的任一个(例如氨基酸残基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或134)。

182.权利要求178中任一项的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

183.权利要求178-182的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

184.权利要求178-182的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸21-123有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

185.权利要求178-182的异多聚体，其中所述ALK2多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:18的氨基酸21-35的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29、30、31、32、33、34和35)，和

b)止于SEQ ID NO:18的氨基酸99-123的任一个(例如氨基酸残基99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122和123)。

186.权利要求178-182的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

187.一种包含ALK3多肽和ActRIIB多肽的重组异多聚体。

188.权利要求187的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

189.权利要求187的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-131有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

190.权利要求187的异多聚体，其中所述ActRIIB多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:1的氨基酸20-29的任一个(例如氨基酸残基20、21、22、23、24、25、26、27、28或29)，和

b)止于SEQ ID NO:1的氨基酸109-134的任一个(例如氨基酸残基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或134)。

191.权利要求187中任一项的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

192.权利要求187-190的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸61-130有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

193.权利要求187-190的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸24-152有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

194.权利要求187-190的异多聚体，其中所述ALK3多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:22的氨基酸24-61的任一个(例如氨基酸残基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和61)，和

b)止于SEQ ID NO:22的氨基酸130-152的任一个(例如氨基酸残基130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151和152)。

195.权利要求187-190的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

196.一种包含ALK4多肽和ActRIIB多肽的重组异多聚体。

197.权利要求196的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

198.权利要求196的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-

131有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

199.权利要求196的异多聚体，其中所述ActRIIB多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:1的氨基酸20-29的任一个(例如氨基酸残基20、21、22、23、24、25、26、27、28或29)，和

b)止于SEQ ID NO:1的氨基酸109-134的任一个(例如氨基酸残基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或134)。

200.权利要求196中任一项的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

201.权利要求196-200的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

202.权利要求130-133的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸23-126有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

203.权利要求196-200的异多聚体，其中所述ALK4多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:26或83的氨基酸23-34的任一个(例如氨基酸残基23、24、25、26、27、28、29、30、31、32、33、34)，和

b)止于SEQ ID NO:26或83的氨基酸101-126的任一个(例如氨基酸残基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和126)。

204.权利要求196-200的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

205.一种包含ALK5多肽和ActRIIB多肽的重组异多聚体。

206.权利要求205的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

207.权利要求205的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-131有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

208.权利要求205的异多聚体，其中所述ActRIIB多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:1的氨基酸20-29的任一个(例如氨基酸残基20、21、22、23、24、25、26、27、28或29)，和

b)止于SEQ ID NO:1的氨基酸109-134的任一个(例如氨基酸残基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或134)。

209.权利要求205中任一项的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

210.权利要求205-209的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸36-106有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

211.权利要求205-209的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸25-126有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

212.权利要求205-209的异多聚体，其中所述ALK5多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:30或87的氨基酸25-36的任一个(例如氨基酸残基25、26、27、28、29、30、31、32、33、34、35和36)，和

b)止于SEQ ID NO:30或87的氨基酸106-126的任一个(例如氨基酸残基106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

213.权利要求205-209的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

214.一种包含ALK6多肽和ActRIIB多肽的重组异多聚体。

215.权利要求214的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

216.权利要求214的异多聚体，其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-131有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

217.权利要求214的异多聚体，其中所述ActRIIB多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:1的氨基酸20-29的任一个(例如氨基酸残基20、21、22、23、24、25、26、27、28或29),和

b)止于SEQ ID NO:1的氨基酸109-134的任一个(例如氨基酸残基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或134)。

218.权利要求214中任一项的异多聚体,其中所述ActRIIB多肽包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

219.权利要求214-218的异多聚体,其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸32-102有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

220.权利要求214-218的异多聚体,其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸14-126有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

221.权利要求214-218的异多聚体,其中所述ALK6多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32),和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

222.权利要求214-218的异多聚体,其中所述ALK6多肽包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

223.权利要求214-218的异多聚体,其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸62-132有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

224.权利要求214-218的异多聚体,其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸26-156有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

225.权利要求214-218的异多聚体,其中所述ALK6多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32),和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、

106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

226.一种包含ALK7多肽和ActRIIB多肽的重组异多聚体。

227.权利要求226的异多聚体,其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸29-109有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

228.权利要求226的异多聚体,其中所述ActRIIB多肽包含与SEQ ID NO:1的氨基酸25-131有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

229.权利要求226的异多聚体,其中所述ActRIIB多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:1的氨基酸20-29的任一个(例如氨基酸残基20、21、22、23、24、25、26、27、28或29),和

b)止于SEQ ID NO:1的氨基酸109-134的任一个(例如氨基酸残基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或134)。

230.权利要求226中任一项的异多聚体,其中所述ActRIIB多肽包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

231.权利要求226-230的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸28-92有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

232.权利要求226-230的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸21-133有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

233.权利要求226-230的异多聚体,其中所述ALK7多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:38、305或309的氨基酸21-28的任一个(例如氨基酸残基21、22、23、24、25、26、27和28),和

b)止于SEQ ID NO:38、305或309的氨基酸92-133的任一个(例如氨基酸残基92、93、94、95、96、97、98、99、100、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、126、127、128、129、130、131、132和133)。

234.权利要求226-230的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、

98%、99%或100%同一性的氨基酸序列。

235.一种包含ALK1多肽和BMPRII多肽的重组异多聚体。

236.权利要求235的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸34-123有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

237.权利要求235的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸27-150有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

238.权利要求235的异多聚体，其中所述BMPRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:46或71的氨基酸27-34的任一个(例如氨基酸残基27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:46或71的氨基酸123-150的任一个(例如氨基酸残基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和150)。

239.权利要求235中任一项的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

240.权利要求235-239的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸34-95有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

241.权利要求235-239的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸22-118有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

242.权利要求235-239的异多聚体，其中所述ALK1多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:14的氨基酸22-34的任一个(例如氨基酸残基22、23、24、25、26、27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:14的氨基酸95-118的任一个(例如氨基酸残基95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117和118)。

243.权利要求235-239的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

244.一种包含ALK2多肽和BMPRII多肽的重组异多聚体。

245.权利要求244的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

246.权利要求244的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸27-150有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

247.权利要求244的异多聚体，其中所述BMPRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:46或71的氨基酸27-34的任一个(例如氨基酸残基27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:46或71的氨基酸123-150的任一个(例如氨基酸残基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和150)。

248.权利要求244中任一项的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

249.权利要求244-248的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

250.权利要求244-248的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸21-123有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

251.权利要求244-248的异多聚体，其中所述ALK2多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:18的氨基酸21-35的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29、30、31、32、33、34和35)，和

b)止于SEQ ID NO:18的氨基酸99-123的任一个(例如氨基酸残基99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122和123)。

252.权利要求244-248的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

253.一种包含ALK3多肽和BMPRII多肽的重组异多聚体。

254.权利要求253的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、

94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

255.权利要求253的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸27-150有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

256.权利要求253的异多聚体，其中所述BMPRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:46或71的氨基酸27-34的任一个(例如氨基酸残基27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:46或71的氨基酸123-150的任一个(例如氨基酸残基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和150)。

257.权利要求253中任一项的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

258.权利要求253-257的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸61-130有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

259.权利要求253-257的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸24-152有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

260.权利要求253-257的异多聚体，其中所述ALK3多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:22的氨基酸24-61的任一个(例如氨基酸残基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和61)，和

b)止于SEQ ID NO:22的氨基酸130-152的任一个(例如氨基酸残基130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151和152)。

261.权利要求253-257的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

262.一种包含ALK4多肽和BMPRII多肽的重组异多聚体。

263.权利要求262的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸34-123有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

264.权利要求262的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸27-150有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

265.权利要求262的异多聚体，其中所述BMPRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:46或71的氨基酸27-34的任一个(例如氨基酸残基27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:46或71的氨基酸123-150的任一个(例如氨基酸残基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和150)。

266.权利要求262中任一项的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

267.权利要求262-266的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

268.权利要求262-266的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸23-126有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

269.权利要求262-266的异多聚体，其中所述ALK4多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:26或83的氨基酸23-34的任一个(例如氨基酸残基23、24、25、26、27、28、29、30、31、32、33、34)，和

b)止于SEQ ID NO:26或83的氨基酸101-126的任一个(例如氨基酸残基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和126)。

270.权利要求262-266的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

271.一种包含ALK5多肽和BMPRII多肽的重组异多聚体。

272.权利要求271的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

273.权利要求271的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸27-150有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、

94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

274.权利要求271的异多聚体，其中所述BMPRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:46或71的氨基酸27-34的任一个(例如氨基酸残基27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:46或71的氨基酸123-150的任一个(例如氨基酸残基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和150)。

275.权利要求271中任一项的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

276.权利要求271-275的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸36-106有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

277.权利要求271-275的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸25-126有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

278.权利要求271-275的异多聚体，其中所述ALK5多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:30或87的氨基酸25-36的任一个(例如氨基酸残基25、26、27、28、29、30、31、32、33、34、35和36)，和

b)止于SEQ ID NO:30或87的氨基酸106-126的任一个(例如氨基酸残基106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

279.权利要求271-275的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

280.一种包含ALK6多肽和BMPRII多肽的重组异多聚体。

281.权利要求280的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸34-123有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

282.权利要求280的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸27-150有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

283.权利要求280的异多聚体，其中所述BMPRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、

98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:46或71的氨基酸27-34的任一个(例如氨基酸残基27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:46或71的氨基酸123-150的任一个(例如氨基酸残基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和150)。

284.权利要求280中任一项的异多聚体，其中所述BMPRII多肽包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

285.权利要求280-284的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸32-102有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

286.权利要求280-284的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸14-126有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

287.权利要求280-284的异多聚体，其中所述ALK6多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32)，和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

288.权利要求280-284的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

289.权利要求280-284的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸62-132有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

290.权利要求280-284的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸26-156有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

291.权利要求280-284的异多聚体，其中所述ALK6多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32)，和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

292.一种包含ALK7多肽和BMPRII多肽的重组异多聚体。

293.权利要求280的异多聚体,其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸34-123有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

294.权利要求292的异多聚体,其中所述BMPRII多肽包含与SEQ ID NO:46或71的氨基酸27-150有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

295.权利要求292的异多聚体,其中所述BMPRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:46或71的氨基酸27-34的任一个(例如氨基酸残基27、28、29、30、31、32、33和34),和

b)止于SEQ ID NO:46或71的氨基酸123-150的任一个(例如氨基酸残基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和150)。

296.权利要求292中任一项的异多聚体,其中所述BMPRII多肽包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

297.权利要求292-296的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸28-92有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

298.权利要求292-296的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸21-133有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

299.权利要求292-296的异多聚体,其中所述ALK7多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:38、305或309的氨基酸21-28的任一个(例如氨基酸残基21、22、23、24、25、26、27和28),和

b)止于SEQ ID NO:38、305或309的氨基酸92-133的任一个(例如氨基酸残基92、93、94、95、96、97、98、99、100、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、126、127、128、129、130、131、132和133)。

300.权利要求292-296的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的任一个有至少70%、

75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

301.一种包含ALK1多肽和TGFBRII多肽的重组异多聚体。

302.权利要求301的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸44-163有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

303.权利要求301的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸23-191有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

304.权利要求301的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

305.权利要求301的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸51-143有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

306.权利要求301的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸23-166有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

307.权利要求301的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

308.权利要求301中任一项的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

309.权利要求301-308的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸34-95有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

310.权利要求301-308的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸

22-118有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

311.权利要求301-308的异多聚体，其中所述ALK1多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:14的氨基酸22-34的任一个(例如氨基酸残基22、23、24、25、26、27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:14的氨基酸95-118的任一个(例如氨基酸残基95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117和118)。

312.权利要求301-308的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

313.一种包含ALK2多肽和TGFBRII多肽的重组异多聚体。

314.权利要求313的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸44-163有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

315.权利要求313的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸23-191有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

316.权利要求313的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

317.权利要求313的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

318.权利要求313的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸23-166有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

319.权利要求313的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、

32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

320.权利要求313中任一项的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

321.权利要求313-320的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

322.权利要求313-320的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸21-123有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

323.权利要求313-320的异多聚体，其中所述ALK2多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:18的氨基酸21-35的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29、30、31、32、33、34和35)，和

b)止于SEQ ID NO:18的氨基酸313-320的任一个(例如氨基酸残基99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122和123)。

324.权利要求244-248的异多聚体，其中所述ALK2多肽包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

325.一种包含ALK3多肽和TGFBRII多肽的重组异多聚体。

326.权利要求325的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸44-163有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

327.权利要求325的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸23-191有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

328.权利要求325的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

329.权利要求325的异多聚体,其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸51-143有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

330.权利要求325的异多聚体,其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸23-166有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

331.权利要求325的异多聚体,其中所述TGFBRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44),和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

332.权利要求325中任一项的异多聚体,其中所述TGFBRII多肽包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

333.权利要求325-332的异多聚体,其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸61-130有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

334.权利要求325-332的异多聚体,其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸24-152有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

335.权利要求325-332的异多聚体,其中所述ALK3多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:22的氨基酸24-61的任一个(例如氨基酸残基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和61),和

b)止于SEQ ID NO:22的氨基酸130-152的任一个(例如氨基酸残基130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151和152)。

336.权利要求325-332的异多聚体,其中所述ALK3多肽包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

337.一种包含ALK4多肽和TGFBRII多肽的重组异多聚体。

338.权利要求337的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸44-163有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

339.权利要求337的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸23-191有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

340.权利要求337的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

341.权利要求337的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

342.权利要求337的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸23-166有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

343.权利要求337的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

345.权利要求337中任一项的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

346.权利要求337-345的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

347.权利要求337-345的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸23-126有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

348.权利要求337-345的异多聚体，其中所述ALK4多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:26或83的氨基酸23-34的任一个(例如氨基酸残基23、24、25、26、27、28、29、30、31、32、33、34)，和

b)止于SEQ ID NO:26或83的氨基酸101-126的任一个(例如氨基酸残基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和126)。

349.权利要求337-345的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

350.一种包含ALK5多肽和TGFBRII多肽的重组异多聚体。

351.权利要求350的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸44-163有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

352.权利要求350的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸23-191有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

353.权利要求350的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

354.权利要求350的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

355.权利要求350的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸23-166有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

356.权利要求350的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

357.权利要求350中任一项的异多聚体,其中所述TGFBRII多肽包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

358.权利要求350-357的异多聚体,其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸36-106有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

359.权利要求350-357的异多聚体,其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸25-126有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

360.权利要求350-357的异多聚体,其中所述ALK5多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:30或87的氨基酸25-36的任一个(例如氨基酸残基25、26、27、28、29、30、31、32、33、34、35和36),和

b)止于SEQ ID NO:30或87的氨基酸106-126的任一个(例如氨基酸残基106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

361.权利要求350-357的异多聚体,其中所述ALK5多肽包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

362.一种包含ALK6多肽和TGFBRII多肽的重组异多聚体。

363.权利要求362的异多聚体,其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸44-163有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

364.权利要求362的异多聚体,其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸23-191有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

365.权利要求362的异多聚体,其中所述TGFBRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44),和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

366.权利要求362的异多聚体,其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸

51-143有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

367.权利要求362的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸23-166有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

368.权利要求362的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

369.权利要求362中任一项的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

370.权利要求362-369的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸32-102有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

371.权利要求362-369的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸14-126有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

372.权利要求362-369的异多聚体，其中所述ALK6多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32)，和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

373.权利要求362-369的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

374.权利要求362-369的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

375.权利要求362-369的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸

26-156有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

376.权利要求362-369的异多聚体，其中所述ALK6多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32)，和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

377.一种包含ALK7多肽和TGFBRII多肽的重组异多聚体。

378.权利要求377的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸44-163有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

379.权利要求377的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:67的氨基酸23-191有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

380.权利要求377的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

381.权利要求377的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

382.权利要求377的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42的氨基酸23-166有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

383.权利要求377的异多聚体，其中所述TGFBRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:67的氨基酸23-44的任一个(例如23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44)，和

b)止于SEQ ID NO:67的氨基酸163-191的任一个(例如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或191)。

384.权利要求377中任一项的异多聚体，其中所述TGFBRII多肽包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

385.权利要求377-384的异多聚体，其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸28-92有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

386.权利要求377-384的异多聚体，其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸21-133有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

387.权利要求377-384的异多聚体，其中所述ALK7多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:38、305或309的氨基酸21-28的任一个(例如氨基酸残基21、22、23、24、25、26、27和28)，和

b)止于SEQ ID NO:38、305或309的氨基酸92-133的任一个(例如氨基酸残基92、93、94、95、96、97、98、99、100、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、126、127、128、129、130、131、132和133)。

388.权利要求377-384的异多聚体，其中所述ALK7多肽包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

389.一种包含ALK1多肽和MISRII多肽的重组异多聚体。

390.权利要求389的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸24-116有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

391.权利要求389的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸17-149有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

392.权利要求389的异多聚体，其中所述MISRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:50、75或79的氨基酸17-24的任一个(例如氨基酸残基17、18、19、20、21、22、23和24)，和

b)止于SEQ ID NO:50、75或79的氨基酸116-149的任一个(例如氨基酸残基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和149)。

393.权利要求389中任一项的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、

51、75、76、79、80、133、135、161、162、419、420、457和458的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

394.权利要求389-394的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸34-95有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

395.权利要求389-394的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14的氨基酸22-118有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

396.权利要求389-394的异多聚体，其中所述ALK1多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:14的氨基酸22-34的任一个(例如氨基酸残基22、23、24、25、26、27、28、29、30、31、32、33和34)，和

b)止于SEQ ID NO:14的氨基酸95-118的任一个(例如氨基酸残基95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117和118)。

397.权利要求389-394的异多聚体，其中所述ALK1多肽包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

398.一种包含ALK2多肽和MISRII多肽的重组异多聚体。

399.权利要求398的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

400.权利要求398的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸17-149有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

401.权利要求398的异多聚体，其中所述MISRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:50、75或79的氨基酸17-24的任一个(例如氨基酸残基17、18、19、20、21、22、23和24)，和

b)止于SEQ ID NO:50、75或79的氨基酸116-149的任一个(例如氨基酸残基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和149)。

402.权利要求398中任一项的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或

100%同一性的氨基酸序列。

403.权利要求398-402的异多聚体,其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸35-99有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

404.权利要求398-402的异多聚体,其中所述ALK2多肽包含与SEQ ID NO:18的氨基酸21-123有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

405.权利要求398-402的异多聚体,其中所述ALK2多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:18的氨基酸21-35的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29、30、31、32、33、34和35),和

b)止于SEQ ID NO:18的氨基酸99-123的任一个(例如氨基酸残基99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122和123)。

406.权利要求398-402的异多聚体,其中所述ALK2多肽包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

407.一种包含ALK3多肽和MISRII多肽的重组异多聚体。

408.权利要求407的异多聚体,其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

409.权利要求407的异多聚体,其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸17-149有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

410.权利要求407的异多聚体,其中所述MISRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:50、75或79的氨基酸17-24的任一个(例如氨基酸残基17、18、19、20、21、22、23和24),和

b)止于SEQ ID NO:50、75或79的氨基酸116-149的任一个(例如氨基酸残基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和149)。

411.权利要求407中任一项的异多聚体,其中所述MISRII多肽包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

412.权利要求407-411的异多聚体,其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸

61-130有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

413.权利要求407-411的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22的氨基酸24-152有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

414.权利要求407-411的异多聚体，其中所述ALK3多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:22的氨基酸24-61的任一个(例如氨基酸残基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和61)，和

b)止于SEQ ID NO:22的氨基酸130-152的任一个(例如氨基酸残基130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151和152)。

415.权利要求407-411的异多聚体，其中所述ALK3多肽包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

416.一种包含ALK4多肽和MISRII多肽的重组异多聚体。

417.权利要求416的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸24-116有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

418.权利要求416的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸17-149有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

419.权利要求416的异多聚体，其中所述MISRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:50、75或79的氨基酸17-24的任一个(例如氨基酸残基17、18、19、20、21、22、23和24)，和

b)止于SEQ ID NO:50、75或79的氨基酸116-149的任一个(例如氨基酸残基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和149)。

420.权利要求416中任一项的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

421.权利要求416-420的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、

94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

422.权利要求416-420的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26或83的氨基酸23-126有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

423.权利要求416-420的异多聚体，其中所述ALK4多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:26或83的氨基酸23-34的任一个(例如氨基酸残基23、24、25、26、27、28、29、30、31、32、33、34)，和

b)止于SEQ ID NO:26或83的氨基酸101-126的任一个(例如氨基酸残基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和126)。

424.权利要求416-420的异多聚体，其中所述ALK4多肽包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

425.一种包含ALK5多肽和MISRII多肽的重组异多聚体。

426.权利要求425的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

427.权利要求425的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸17-149有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

428.权利要求425的异多聚体，其中所述MISRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:50、75或79的氨基酸17-24的任一个(例如氨基酸残基17、18、19、20、21、22、23和24)，和

b)止于SEQ ID NO:50、75或79的氨基酸116-149的任一个(例如氨基酸残基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和149)。

429.权利要求425中任一项的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

430.权利要求425-429的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30或87的氨基酸36-106有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

431.权利要求425-429的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30或87的氨

基酸25-126有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

432.权利要求425-429的异多聚体，其中所述ALK5多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:30或87的氨基酸25-36的任一个(例如氨基酸残基25、26、27、28、29、30、31、32、33、34、35和36)，和

b)止于SEQ ID NO:30或87的氨基酸106-126的任一个(例如氨基酸残基106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

433.权利要求425-429的异多聚体，其中所述ALK5多肽包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

434.一种包含ALK6多肽和MISRII多肽的重组异多聚体。

435.权利要求434的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸24-116有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

436.权利要求434的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸17-149有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

437.权利要求434的异多聚体，其中所述MISRII多肽包含与下列多肽有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，所述多肽：

a)始于SEQ ID NO:50、75或79的氨基酸17-24的任一个(例如氨基酸残基17、18、19、20、21、22、23和24)，和

b)止于SEQ ID NO:50、75或79的氨基酸116-149的任一个(例如氨基酸残基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和149)。

438.权利要求434中任一项的异多聚体，其中所述MISRII多肽包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458的任一个有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

439.权利要求280-284的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸32-102有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

440.权利要求434-439的异多聚体，其中所述ALK6多肽包含与SEQ ID NO:34的氨基酸14-126有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

441.权利要求434-439的异多聚体，其中所述ALK6多肽包含与下列多肽有至少70％、

75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32),和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

442.权利要求434-439的异多聚体,其中所述ALK6多肽包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

443.权利要求434-439的异多聚体,其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸62-132有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

444.权利要求434-439的异多聚体,其中所述ALK6多肽包含与SEQ ID NO:91的氨基酸26-156有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

445.权利要求434-439的异多聚体,其中所述ALK6多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32),和

b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)。

446.一种包含ALK7多肽和MISRII多肽的重组异多聚体。

447.权利要求446的异多聚体,其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

448.权利要求446的异多聚体,其中所述MISRII多肽包含与SEQ ID NO:50、75或79的氨基酸17-149有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

449.权利要求446的异多聚体,其中所述MISRII多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:50、75或79的氨基酸17-24的任一个(例如氨基酸残基17、18、19、20、21、22、23和24),和

b)止于SEQ ID NO:50、75或79的氨基酸116-149的任一个(例如氨基酸残基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和149)。

450.权利要求446中任一项的异多聚体,其中所述MISRII多肽包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

451.权利要求446-450的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸28-92有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

452.权利要求446-450的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、305或309的氨基酸21-133有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

453.权利要求446-450的异多聚体,其中所述ALK7多肽包含与下列多肽有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,所述多肽:

a)始于SEQ ID NO:38、305或309的氨基酸21-28的任一个(例如氨基酸残基21、22、23、24、25、26、27和28),和

b)止于SEQ ID NO:38、305或309的氨基酸92-133的任一个(例如氨基酸残基92、93、94、95、96、97、98、99、100、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、126、127、128、129、130、131、132和133)。

454.权利要求446-450的异多聚体,其中所述ALK7多肽包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的任一个有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

455.一种包含ALK1多肽和至少一种其它的TGF-β I型受体多肽的重组异多聚体,其中所述至少一种其它的TGF-β I型受体多肽不是ALK1多肽。

456.权利要求455的异多聚体,其中所述ALK1多肽包含与本文公开的ALK1序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

457.权利要求455或456的异多聚体,其中所述不是ALK1多肽的至少一种其它的TGF-β I型受体多肽选自:ALK2多肽、ALK3多肽、ALK4多肽、ALK5多肽、ALK6多肽和ALK7多肽。

458.权利要求457的异多聚体,其中所述不是ALK1多肽的至少一种其它的TGF-β I型受体多肽选自:

a)包含与本文公开的ALK2序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列的ALK2多肽,

b)包含与本文公开的ALK3序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列的ALK3多肽,

c)包含与本文公开的ALK4序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK4多肽，

d)包含与本文公开的ALK5序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK5多肽，

e)包含与本文公开的ALK6序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK6多肽，

f)包含与本文公开的ALK7序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK7多肽。

459.一种包含ALK2多肽和至少一种其它的TGF-β I型受体多肽的重组异多聚体，其中所述至少一种其它的TGF-β I型受体多肽不是ALK2多肽。

460.权利要求459的异多聚体，其中所述ALK2多肽包含与本文公开的ALK2序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

461.权利要求459或460的异多聚体，其中所述不是ALK2多肽的至少一种其它的TGF-β I型受体多肽选自：ALK3多肽、ALK4多肽、ALK5多肽、ALK6多肽和ALK7多肽。

462.权利要求461的异多聚体，其中所述不是ALK2多肽的至少一种其它的TGF-β I型受体多肽选自：

a)包含与本文公开的ALK3序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK3多肽，

b)包含与本文公开的ALK4序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK4多肽，

c)包含与本文公开的ALK5序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK5多肽，

d)包含与本文公开的ALK6序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK6多肽，

e)包含与本文公开的ALK7序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK7多肽。

463.一种包含ALK3多肽和至少一种其它的TGF-β I型受体多肽的重组异多聚体，其中所述至少一种其它的TGF-β I型受体多肽不是ALK3多肽。

464.权利要求463的异多聚体，其中所述ALK3多肽包含与本文公开的ALK3序列(例如

SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

465.权利要求463或464的异多聚体，其中所述不是ALK3多肽的至少一种其它的TGF-β I型受体多肽选自：ALK4多肽、ALK5多肽、ALK6多肽和ALK7多肽。

466.权利要求465的异多聚体，其中所述不是ALK3多肽的至少一种其它的TGF-β I型受体多肽选自：

a)包含与本文公开的ALK4序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK4多肽，

b)包含与本文公开的ALK5序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK5多肽，

c)包含与本文公开的ALK6序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK6多肽，

d)包含与本文公开的ALK7序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK7多肽。

467.一种包含ALK4多肽和至少一种其它的TGF-β I型受体多肽的重组异多聚体，其中所述至少一种其它的TGF-β I型受体多肽不是ALK4多肽。

468.权利要求467的异多聚体，其中所述ALK4多肽包含与本文公开的ALK4序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

469.权利要求467或468的异多聚体，其中所述不是ALK4多肽的至少一种其它的TGF-β I型受体多肽选自：ALK5多肽、ALK6多肽和ALK7多肽。

470.权利要求469的异多聚体，其中所述不是ALK4多肽的至少一种其它的TGF-β I型受体多肽选自：

a)包含与本文公开的ALK5序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK5多肽，

b)包含与本文公开的ALK6序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK6多肽，

c)包含与本文公开的ALK7序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的ALK7多肽。

471.一种包含ALK5多肽和至少一种其它的TGF-β I型受体多肽的重组异多聚体，其中所述至少一种其它的TGF-β I型受体多肽不是ALK5多肽。

472.权利要求471的异多聚体，其中所述ALK5多肽包含与本文公开的ALK5序列(例如

SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

473.权利要求471或472的异多聚体,其中所述不是ALK5多肽的至少一种其它的TGF-β I型受体多肽选自:ALK6多肽和ALK7多肽。

474.权利要求473的异多聚体,其中所述不是ALK5多肽的至少一种其它的TGF-β I型受体多肽选自:

a)包含与本文公开的ALK6序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列的ALK6多肽,

b)包含与本文公开的ALK7序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列的ALK7多肽。

475.一种包含ALK6多肽和至少一种其它的TGF-β I型受体多肽的重组异多聚体,其中所述至少一种其它的TGF-β I型受体多肽不是ALK6多肽。

476.权利要求475的异多聚体,其中所述ALK6多肽包含与本文公开的ALK6序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

477.权利要求475或476的异多聚体,其中所述不是ALK6多肽的至少一种其它的TGF-β I型受体多肽是ALK7多肽。

478.权利要求477的异多聚体,ALK7多肽包含与本文公开的ALK7序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

479.一种包含ActRIIA多肽和至少一种其它的TGF-β II型受体多肽的重组异多聚体,其中所述至少一种其它的TGF-β II型受体多肽不是ActRIIA多肽。

480.权利要求479的异多聚体,其中所述ActRIIA多肽包含与本文公开的ActRIIA序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

481.权利要求479或480的异多聚体,其中所述不是ActRIIA多肽的至少一种其它的TGF-β II型受体多肽选自:ActRIIB多肽、BMPRII多肽、TGFBRII多肽和MISRII多肽。

482.权利要求481的异多聚体,其中所述不是ActRIIA多肽的至少一种其它的TGF-β II型受体多肽选自:

a)包含与本文公开的ActRIIB序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列的ActRIIB多肽,

b)包含与本文公开的BMPRII序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列的BMPRII多肽,

c)包含与本文公开的TGFBRII序列(例如SEQ ID NO)有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%

同一性的氨基酸序列的TGFBRII多肽，

d)包含与本文公开的MISRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的MISRII多肽。

483.一种包含ActRIIB多肽和至少一种其它的TGF-β II型受体多肽的重组异多聚体，其中所述至少一种其它的TGF-β II型受体多肽不是ActRIIB多肽。

484.权利要求483的异多聚体，其中所述ActRIIB多肽包含与本文公开的ActRIIB序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

485.权利要求体483或484的异多聚体，其中所述不是ActRIIB多肽的至少一种其它的TGF-β II型受体多肽选自：BMPRII多肽、TGFBRII多肽和MISRII多肽。

486.权利要求485的异多聚体，其中所述不是ActRIIB多肽的至少一种其它的TGF-β II型受体多肽选自：

b)包含与本文公开的BMPRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的BMPRII多肽，

c)包含与本文公开的TGFBRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的TGFBRII多肽，

d)包含与本文公开的MISRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的MISRII多肽。

487.一种包含BMPRII多肽和至少一种其它的TGF-β II型受体多肽的重组异多聚体，其中所述至少一种其它的TGF-β II型受体多肽不是BMPRII多肽。

488.权利要求487的异多聚体，其中所述BMPRII多肽包含与本文公开的BMPRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

489.权利要求487或488的异多聚体，其中所述不是BMPRII多肽的至少一种其它的TGF-β II型受体多肽选自：TGFBRII多肽和MISRII多肽。

490.权利要求489的异多聚体，其中所述不是BMPRII多肽的至少一种其它的TGF-β II型受体多肽选自：

a)包含与本文公开的TGFBRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的TGFBRII多肽，

b)包含与本文公开的MISRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的MISRII多肽。

491.一种包含TGFBRII多肽和至少一种其它的TGF-β II型受体多肽的重组异多聚体，其中所述至少一种其它的TGF-β II型受体多肽不是TGFBRII多肽。

492.权利要求491的异多聚体，其中所述TGFBRII多肽包含与本文公开的TGFBRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

493.权利要求491或492的异多聚体，其中所述不是TGFBRII多肽的至少一种其它的TGF-β II型受体多肽是MISRII多肽。

494.权利要求493的异多聚体，其中所述MISRII多肽包含与本文公开的MISRII序列(例如SEQ ID NO)有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列。

495.一种描述于表:“II型:II型受体异二聚体”中的重组异多聚体。

460.一种描述于表:“I型:I型受体异二聚体”中的重组异多聚体。

461.一种描述于表:“I型:II型受体异二聚体”中的重组异多聚体。

TGF-β超家族I型和II型受体异多聚体及其用途

[0001] 相关申请的交叉引用

[0002] 本申请要求2015年4月6日提交的美国临时申请顺序号62/143,579的优选权的权益。前述申请的公开内容通过引用以其整体结合到本文中。

[0003] 发明背景

[0004] 转化生长因子-β(TGF-β)超家族含有多种具有共同的序列元件和结构基序的生长因子。已知这些蛋白质在脊椎动物和无脊椎动物两者中对多种细胞类型发挥生物作用。该超家族的成员在图式形成和组织特化的胚胎发育期间发挥重要功能,并可影响各种分化过程,包括脂肪形成、肌发生、软骨发生、心脏发生、血细胞生成、神经发生和上皮细胞分化。该家族被分成两个通用系统分化进化枝:该超家族的较新进化的成员(这包括TGF-β、激活素和nodal)和该超家族较远缘的蛋白质的节点和进化枝(这包括多种BMP和GDF)。Hinck(2012)FEBS Letters586:1860-1870。TGF-β家族成员不同的常常互补的生物作用。通过操控TGF-β家族成员的活性,常常可引起生物体的重大生理改变。例如Piedmontese和Belgian Blue牛品种在GDF8(亦称肌肉生长抑制素)基因中携带引起肌肉质量明显增加的功能缺失突变。Grobet等(1997)Nat Genet.,17(1):71-4。此外,在人中,GDF8的无活性等位基因与肌肉质量增加和据报道的异常强度有关。Schuelke等(2004)N Engl J Med,350:2682-8。

[0005] 可通过提高或抑制由TGF-β家族的配体介导的信号转导(例如SMAD1、2、3、5和/或8)实现肌肉、骨、脂肪、红细胞和其它组织的改变。因此,需要调节TGF-β超家族的不同配体的活性的作用剂。

[0006] 发明概述

[0007] 一方面,本公开内容提供包含至少一种TGF-β超家族I型丝氨酸/苏氨酸激酶受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7多肽)(包括其片段和变体)和至少一种TGF-β超家族II型丝氨酸/苏氨酸激酶受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII)(包括其片段和变体)的异多聚体复合体(heteromultimeric complex)。另一方面,本公开内容提供包含至少两种不同的TGF-β超家族I型丝氨酸/苏氨酸激酶受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7多肽)(包括其片段和变体)的异多聚体复合体。又一方面,本公开内容提供包含至少两种不同的TGF-β超家族II型丝氨酸/苏氨酸激酶受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII)(包括其片段和变体)的异多聚体复合体。任选,与其相应的同多聚复合体(例如ActRIIB同二聚体和ALK4同二聚体)相比,本文公开的异多聚体复合体(例如ActRIIB:ALK4异二聚体)具有不同的配体结合特异性/特征。如本文实施例所示,包含TGF-β超家族的I型和II型受体多肽的异源多聚多肽复合体显示新的性质,包括新的配体结合特性。

[0008] 异多聚体结构包括例如异二聚体、异三聚体和更高阶的复合体。参见例如图1、2、15、16、17、18、19。在一些实施方案中,本公开内容的异多聚体是异二聚体。优选本文所述TGF-β超家族I型和II型受体多肽包含受体的配体结合域,例如TGF-β超家族I型或II型受体的胞外域。因此,在某些方面,本文所述蛋白质复合体包含选自以下的II型TGF-β超家族受体的胞外域:ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII及其截短物和变体,以及选自以

下的I型TGF-β超家族受体的胞外域:ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7及其截短物和变体。优选本文所述TGF-β超家族I型和II型多肽,以及包含所述多肽的蛋白质复合体是可溶解的。在某些方面,本公开内容的异多聚体复合体与一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、缪勒抑制物质(MIS)和Lefty)结合。任选本公开内容的蛋白质复合体以大于或等于10^{-8}、10^{-9}、10^{-10}、10^{-11}或10^{-12}的K_D与这些配体的一种或多种结合。一般而言,本公开内容的异多聚体拮抗(抑制)至少一种TGF-β超家族配体的一种或多种活性,而且活性的这类改变可采用本领域已知的各种测定法测量,包括例如如本文所述基于细胞的测定法。优选本公开内容的异多聚体在哺乳动物(例如小鼠或人)中显示至少4、6、12、24、36、48或72小时的血清半寿期。任选本公开内容的异多聚体在哺乳动物(例如小鼠或人)中可显示至少6、8、10、12、14、20、25或30天的血清半寿期。

[0009] 在某些方面,本文所述异多聚体包含与第二多肽共价或非共价缔合的第一多肽,其中第一多肽包含TGF-β超家族I型受体多肽的氨基酸序列和相互作用对的第一成员的氨基酸序列,第二多肽包含TGF-β超家族II型受体多肽的氨基酸序列和相互作用对的第二成员的氨基酸序列。另一方面,本文所述异多聚体包含与第二多肽共价或非共价缔合的第一多肽,其中第一多肽包含TGF-β超家族I型受体多肽的氨基酸序列和相互作用对的第一成员的氨基酸序列,第二多肽包含不同TGF-β超家族I型受体多肽的氨基酸序列和相互作用对的第二成员的氨基酸序列。又一方面,本文所述异多聚体包含与第二多肽共价或非共价缔合的第一多肽,其中第一多肽包含TGF-β超家族II型受体多肽的氨基酸序列和相互作用对的第一成员的氨基酸序列,第二多肽包含不同TGF-β超家族II型受体多肽的氨基酸序列和相互作用对的第二成员的氨基酸序列。任选TGF-β超家族I型受体多肽与相互作用对的第一成员直接连接,或间插序列(例如接头)可位于TGF-β超家族I型受体多肽的氨基酸序列和相互作用对的第一成员的氨基酸序列之间。同样,TGF-β超家族II型受体多肽可与相互作用对的第二成员直接连接,或间插序列(例如接头)可位于TGF-β超家族II型受体多肽的氨基酸序列和相互作用对的第二成员的氨基酸序列之间。接头的实例包括但不限于序列TGGG(SEQ ID NO:62)、TGGGG(SEQ ID NO:60)、SGGGG(SEQ ID NO:61)、GGGG(SEQ ID NO:59)和GGG(SEQ ID NO:58)。

[0010] 本文所述相互作用对经设计以促进二聚化或形成更高阶的多聚体。在一些实施方案中,相互作用对可以是相互作用形成复合体、特别是异二聚体复合体的任2个多肽序列,但是有效实施方案也可使用形成同二聚体序列的互作用对。相互作用对的第一或第二成员可以是不对称对,意味着所述对的成员优先彼此缔合而非自身缔合。因此,不对称相互作用对的第一和第二成员可缔合形成异二聚体复合体。或者,相互作用对可为不受引导的(unguided),意味着所述对的成员可彼此缔合或自身缔合而无实质性偏好,因此可具有相同或不同的氨基酸序列。因此,不受引导相互作用对的第一和第二成员可缔合形成同二聚体复合体或异二聚体复合体。任选相互作用对(例如不对称对或不受引导相互作用对)的第一成员与相互作用对的第二成员共价缔合。任选相互作用对(例如不对称对或不受引导相

互作用对)的第一成员与相互作用对的第二成员非共价缔合。任选相互作用对(例如不对称或不受引导相互作用对)的第一成员通过共价和非共价机制两者与相互作用对的第二成员缔合。

[0011] 在一些实施方案中,TGF-β超家族I型受体多肽是包含免疫球蛋白的Fc结构域的融合蛋白。同样,在一些实施方案中,TGF-β超家族II型受体多肽是包含免疫球蛋白的Fc结构域的融合蛋白。传统的Fc融合蛋白和抗体是不受引导相互作用对的实例,而多种工程改造的Fc结构域被设计成不对称相互作用对[Spiess等(2015)Molecular Immunology67(2A):95-106]。因此,本文所述相互作用对的第一成员和/或第二成员可包含免疫球蛋白的恒定结构域,包括例如免疫球蛋白的Fc部分。例如,相互作用对的第一成员可包含来源于IgG(IgG1、IgG2、IgG3或IgG4)、IgA(IgA1或IgA2)、IgE或IgM免疫球蛋白的Fc结构域的氨基酸序列。例如,相互作用对的第一成员可包含与SEQ ID NO:200-214的任一个有至少80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列或基本上由其组成或由其组成。任选相互作用对的第二成员可包含来源于IgG(IgG1、IgG2、IgG3或IgG4)、IgA(IgA1或IgA2)、IgE或IgM的Fc结构域的氨基酸序列。所述免疫球蛋白结构域可包含促进异二聚体形成的一个或多个氨基酸修饰(例如缺失、添加和/或取代)。例如,相互作用对的第二成员可包含与SEQ ID NO:200-214的任一个有至少80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列或基本上由其组成或由其组成。在一些实施方案中,相互作用对的第一成员和第二成员包含来源于相同免疫球蛋白类别或亚类的Fc结构域。在其它实施方案中,相互作用对的第一成员和第二成员包含来源于不同免疫球蛋白类别或亚类的Fc结构域。同样,相互作用对(例如不对称对或不受引导相互作用对)的第一成员和/或第二成员包含修饰的免疫球蛋白的恒定结构域,包括例如修饰的免疫球蛋白的Fc部分。例如,本公开内容的蛋白质复合体可包含第一修饰的免疫球蛋白Fc部分和第二修饰的免疫球蛋白Fc部分,所述第一修饰的Fc部分包含与选自SEQ ID NO:200-214的氨基酸序列有至少80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的氨基酸序列,所述第二修饰的Fc部分可与第一修饰的免疫球蛋白Fc部分的氨基酸序列相同或不同,包含与选自SEQ ID NO:200-214的氨基酸序列有至少80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的氨基酸序列。所述免疫球蛋白结构域可包含促进异二聚体形成的一个或多个氨基酸修饰(例如缺失、添加和/或取代)。

[0012] 在一些实施方案中,本公开内容提供包含I型和II型TGF-β超家族受体多肽的异聚体多肽复合体,其中II型TGF-β超家族受体多肽来源于ActRIIA受体。在一些实施方案中,本公开内容提供包含至少两种不同的II型TGF-β超家族受体多肽的异聚体多肽复合体,其中II型TGF-β超家族受体多肽的至少一种来源于ActRIIA受体。例如,ActRIIA多肽可包含与本文公开的ActRIIA序列(例如SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452)有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列或基本上由其组成或由其组成。任选ActRIIA多肽可包含a)始于SEQ ID NO:9的21-30的氨基酸(例如氨基酸残基21、22、23、24、25、26、27、28、29或30),和b)止于SEQ ID NO:9的氨基酸110-135的任一个(例如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或135)的多肽有至少70%、80%、85%、90%、

95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选本公开内容的ActRIIA多肽可以是另包含对ActRIIA是异源的一个或多个部分(结构域)的融合蛋白。例如,ActRIIA多肽可与包含多聚化结构域的异源多肽融合,任选具有位于ActRIIA多肽和异源多肽之间的接头结构域。在一些实施方案中,本文所述多聚化结构域包含相互作用对的一个组分。在某些方面,包含ActRIIA多肽的异聚体复合体另包含至少一种I型TGF-β超家族受体多肽。例如,ActRIIA异聚体复合体可另包含本文所述ALK1多肽,包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在这个和其它实施方案中,任选ALK1多肽可包含与a)始于SEQ ID NO:14的氨基酸22-34的任一个(例如氨基酸残基22、23、24、25、26、27、28、29、30、31、32、33和34)和b)止于SEQ ID NO:14的氨基酸95-118的任一个(例如氨基酸残基95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117和118)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。在一些实施方案中,ActRIIA异聚体复合体可另包含本文所述ALK2多肽,包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在这个和其它实施方案中,任选ALK2多肽可包含与a)始于SEQ ID NO:18的氨基酸21-35的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29、30、31、32、33、34和35),和b)止于SEQ ID NO:18的氨基酸99-123的任一个(例如氨基酸残基99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122和123)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。在一些实施方案中,ActRIIA异聚体复合体可另包含本文所述ALK3多肽,包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在这个和其它实施方案中,任选ALK3多肽可包含与a)始于SEQ ID NO:22的氨基酸24-61的任一个(例如氨基酸残基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和61),和b)止于SEQ ID NO:22的氨基酸130-152的任一个(例如氨基酸残基130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151和152)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。在一些实施方案中,ActRIIA异聚体复合体可另包含本文所述ALK4多肽,包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在这个和其它实施方中,任选ALK4多肽可包含与a)始于SEQ ID NO:26或83氨基酸23-34的任一个(例如氨基酸残基23、24、25、26、27、28、29、30、31、32、33、34),和b)止于SEQ ID NO:26或83的氨基酸101-126的任一个(例如氨基酸残基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和126)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列

或基本上由其组成或由其组成。在一些实施方案中，ActRIIA异聚体复合体可另包含本文所述ALK5多肽，包括例如包含与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在这个和其它实施方案中，任选ALK5多肽可包含与a)始于SEQ ID NO:30或87的氨基酸25-36的任一个(例如氨基酸残基25、26、27、28、29、30、31、32、33、34、35和36)，和b)止于SEQ ID NO:30或87的氨基酸106-126的任一个(例如氨基酸残基106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。在一些实施方案中，ActRIIA异聚体复合体可另包含本文所述ALK6多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在这个和其它实施方案中，任选ALK6多肽可包含与a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32)，和b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。在这个和其它实施方案中，任选ALK6多肽可包含与a)始于SEQ ID NO:91的氨基酸26-62的任一个(例如氨基酸残基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和62)，和b)止于SEQ ID NO:91的氨基酸132-156的任一个(例如氨基酸残基132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151、152、153、154、155和156)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。在一些实施方案中，ActRIIA异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在这个和其它实施方案中，任选ALK7多肽可包含与始于SEQ ID NO:38的氨基酸21-28的任一个(例如氨基酸21、22、23、24、25、26、27或28)和止于SEQ ID NO:38的氨基酸92-113的任一个(例如SEQ ID NO:38的氨基酸92、93、94、95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112或113)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。在某些方面，包含ActRIIA多肽的异聚体复合体另包含至少一种不同的II型TGF-β超家族受体多肽。例如，ActRIIA异聚体复合体可另包含本文所述ActRIIB多肽，包括例如包含与选自SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ActRIIA异聚体复合体可另包含本文所述BMPRII多肽，包括例如包含与选自SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方

案中，ActRIIA异聚体复合体可另包含本文所述MISRII多肽，包括例如包含与选自SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ActRIIA异聚体复合体可另包含本文所述TGFBRII多肽，包括例如包含与选自SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。

[0013] 在一些实施方案中，本公开内容提供包含I型和II型TGF-β超家族受体多肽的异聚体多肽复合体，其中II型TGF-β超家族受体多肽来源于ActRIIB受体。在一些实施方案中，本公开内容提供包含至少两种不同的II型TGF-β超家族受体多肽的异聚体多肽复合体，其中II型TGF-β超家族受体多肽的至少一种来源于ActRIIB受体。例如，ActRIIB多肽可包含与本文公开的ActRIIB序列(例如SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454)有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选ActRIIB多肽可包含与a)始于SEQ ID NO:1的氨基酸20-29的任一个(例如氨基酸残基20、21、22、23、24、25、26、27、28或29)，和b)止于SEQ ID NO:1的氨基酸109-134的任一个(例如氨基酸残基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或134)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选本公开内容的ActRIIB多肽可以是另包含对于ActRIIB是异源的一个或多个部分(结构域)的融合蛋白。例如，ActRIIB多肽可与包含多聚化结构域的异源多肽融合，任选在ActRIIB多肽和异源多肽之间具有接头结构域。在一些实施方案中，本文所述多聚化结构域包含相互作用对的一个组分。优选包含ActRIIB多肽的异聚体复合体另包含至少一种I型TGF-β超家族受体多肽。例如，ActRIIB异聚体复合体可另包含本文所述ALK1多肽，包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ActRIIB异聚体复合体可另包含本文所述ALK2多肽，包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ActRIIB异聚体复合体可另包含本文所述ALK3多肽，包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ActRIIB异聚体复合体可另包含本文所述ALK4多肽，包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ActRIIB异聚体复合体可另包含本文所述ALK5多肽，包括例如与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ActRIIB异聚体复合体可另包含本文所述ALK6多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、

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[0014] 在一些实施方案中，本公开内容提供包含I型和II型TGF-β超家族受体多肽的异聚体多肽复合体，其中II型TGF-β超家族受体多肽来源于TGFBRII受体。在一些实施方案中，本公开内容提供包含至少两种不同的II型TGF-β超家族受体多肽的异聚体多肽复合体，其中II型TGF-β超家族受体多肽的至少一种来源于TGFBRII受体。例如，TGFBRII多肽可包含与本文公开的TGFBRII序列(例如SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462)有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选TGFBRII多肽可包含与a)始于SEQ ID NO:42的氨基酸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或51的任一个，和b)止于SEQ ID NO:42的氨基酸143、144、145、146、147、148、149、150、151、152、153、154、155、156、157、158、159、160、161、162、163、164、165或166的任一个的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选TGFBRII多肽可包含与a)始于SEQ ID NO:67的氨基酸23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43或44的任一个，和b)止于SEQ ID NO:67的氨基酸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或191的任一个的多肽有至少70％、80％、85％、90％、95％、

97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选本公开内容的TGFBRII多肽可以是另包含对TGFBRII是异源的一个或多个部分(结构域)的融合蛋白。例如,TGFBRII多肽可与包含多聚化结构域的异源多肽融合,任选具有位于TGFBRII多肽和异源多肽之间的接头结构域。在一些实施方案中,本文所述多聚化结构域包含相互作用对的一个组分。优选包含TGFBRII多肽的异聚体复合体另包含至少一种I型TGF-β超家族受体多肽。例如,TGFBRII异聚体复合体可另包含本文所述ALK1多肽,包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,TGFBRII异聚体复合体可另包含本文所述ALK2多肽,包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,TGFBRII异聚体复合体可另包含本文所述ALK3多肽,包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,TGFBRII异聚体复合体可另包含本文所述ALK4多肽,包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,TGFBRII异聚体复合体可另包含本文所述ALK5多肽,包括例如与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,TGFBRII异聚体复合体可另包含本文所述ALK6多肽,包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,TGFBRII异聚体复合体可另包含本文所述ALK7多肽,包括例如包含与选自SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。任选包含TGFBRII多肽的异聚体复合体可另包含一种或多种其它的II型TGF-β超家族受体多肽,包括例如本文所述ActRIIA、ActRIIB、BMPRII和MISRII多肽(例如包含选自SEQ ID NO:1、2、3、4、5、6、9、10、11、46、47、50、51、71、72、75、76、79、80、100、102、118、120、121、123、401、402、409、410、411和412的氨基酸序列、基本由其组成或由其组成的多肽)。在某些方面,包含TGFBRII多肽的异聚体复合体另包含至少一种不同的II型TGF-β超家族受体多肽。例如,TGFBRII异聚体复合体可另包含本文所述ActRIIA多肽,包括例如包含与选自SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,TGFBRII异聚体复合体可另包含本文所述ActRIIB多肽,包括例如包含与选自SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,TGFBRII异聚体复合体可另包含本文所述MISRII多肽,包括例如包

含与选自SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，TGFBRII异聚体复合体可另包含本文所述BMPRII多肽，包括例如包含与选自SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。

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[0017] 在一些实施方案中，本公开内容提供包含至少两种不同的I型TGF-β超家族受体多肽的异聚体多肽复合体，其中I型TGF-β超家族受体多肽的至少一种来源于ALK1受体。例如，ALK1多肽可包含与本文公开的ALK1序列(例如14、15、124、126、171、172、413、414、463和464)有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选ALK1多肽可包含与a)始于SEQ ID NO:14的氨基酸22-34的任一个(例如氨基酸残基22、23、24、25、26、27、28、29、30、31、32、33和34)和b)止于SEQ ID NO:14的氨基酸95-118的任一个(例如氨基酸残基95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117和118)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选本公开内容的ALK1多肽可以是另包含对ALK1是异源的一个或多个部分(结构域)的融合蛋白。例如，ALK1多肽可与包含多聚化结构域的异源多肽融合，任选具有位于之间的接头结构域ALK1多肽和异源多肽。在一些实施方案中，本文所述多聚化结构域包含相互作用对的一个组分。在一些实施方案中，包含ALK1多肽的异聚体复合体另包含至少一种不同的I型TGF-β超家族多肽。例如，ALK1异聚体复合体可另包含本文所述ALK2多肽，包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK1异聚体复合体可另包含本文所述ALK3多肽，包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK1异聚体复合体可另包含本文所述ALK4多肽，包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK1异聚体复合体可另包含本文所述ALK5多肽，包括例如与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK1异聚体复合体可另包含本文所述ALK6多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK1异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK1异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。

[0018] 在一些实施方案中，本公开内容提供包含至少两种不同的I型TGF-β超家族受体多肽的异聚体多肽复合体，其中I型TGF-β超家族受体多肽的至少一种来源于ALK2受体。例如，ALK2多肽可包含与本文公开的ALK2序列(例如18、19、136、138、173、174、421、422、465和

466)有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选ALK2多肽可包含与a)始于SEQ ID NO:18的氨基酸21-35的任一个(例如氨基酸残基21、22、23、24、25、26、27、28、29、30、31、32、33、34和35)，和b)止于SEQ ID NO:18的氨基酸99-123的任一个(例如氨基酸残基99、100、101、102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122和123)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选本公开内容的ALK2多肽可以是另包含对ALK2是异源的一个或多个部分(结构域)的融合蛋白。例如，ALK2多肽可与包含多聚化结构域的异源多肽融合，任选具有位于ALK2多肽和异源多肽之间的接头结构域。在一些实施方案中，本文所述多聚化结构域包含相互作用对的一个组分。在一些实施方案中，包含ALK2多肽的异聚体复合体另包含至少一种不同的I型TGF-β超家族多肽。例如，ALK2异聚体复合体可另包含本文所述ALK1多肽，包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK2异聚体复合体可另包含本文所述ALK3多肽，包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK2异聚体复合体可另包含本文所述ALK4多肽，包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK2异聚体复合体可另包含本文所述ALK5多肽，包括例如与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK2异聚体复合体可另包含本文所述ALK6多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK2异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK2异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。

[0019] 在一些实施方案中，本公开内容提供包含至少两种不同的I型TGF-β超家族受体多肽的异聚体多肽复合体，其中I型TGF-β超家族受体多肽的至少一种来源于ALK3受体。例如，ALK3多肽可包含与本文公开的ALK3序列(例如22、23、115、117、175、176、407、408、467和468)有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选ALK3多肽可包含与a)始于SEQ ID NO:22的氨基酸24-61的任一个(例如氨基酸残基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和61)，和b)止于SEQ ID NO:22的氨基酸130-152的任一个(例如氨基酸残基130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151和152)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选本公开内容的ALK3多肽可以是另包含对ALK3是异源的一个或多个部分(结构域)的融合蛋白。例如，ALK3多肽可与包含多聚化结构域的异源多肽融合，任选具有位于ALK3多肽和异源多肽之间的接头结构域。在一些实施方案中，本文所述多聚化结构域包含相互作用对的一个组分。在一些实施方案中，包含ALK3多肽的异聚体复合体另包含至少一种不同的I型TGF-β超家族多肽。例如，ALK3异聚体复合体可另包含本文所述ALK2多肽，包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK3异聚体复合体可另包含本文所述ALK1多肽，包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK3异聚体复合体可另包含本文所述ALK4多肽，包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK3异聚体复合体可另包含本文所述ALK5多肽，包括例如与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK3异聚体复合体可另包含本文所述ALK6多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK3异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK3异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。

[0020] 在一些实施方案中，本公开内容提供包含至少两种不同的I型TGF-β超家族受体多肽的异聚体多肽复合体，其中I型TGF-β超家族受体多肽的至少一种来源于ALK4受体。例如，ALK4多肽可包含与本文公开的ALK4序列(例如26、27、83、84、104、106、177、178、403、404、469和470)有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选ALK4多肽可包含与a)始于SEQ ID NO:26或83的氨基酸23-34的任一个(例如氨基酸残基23、24、25、26、27、28、29、30、31、32、33、34)，和b)止于SEQ ID NO:26或83的氨基酸101-126的任一个(例如氨基酸残基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和126)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨

基酸序列或基本上由其组成或由其组成。任选本公开内容的ALK4多肽可以是另包含对ALK4是异源的一个或多个部分(结构域)的融合蛋白。例如,ALK4多肽可与包含多聚化结构域的异源多肽融合,任选具有位于ALK4多肽和异源多肽之间的接头结构域。在一些实施方案中,本文所述多聚化结构域包含相互作用对的一个组分。在一些实施方案中,包含ALK4多肽的异聚体复合体另包含至少一种不同的I型TGF-β超家族多肽。例如,ALK4异聚体复合体可另包含本文所述ALK2多肽,包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK4异聚体复合体可另包含本文所述ALK3多肽,包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK4异聚体复合体可另包含本文所述ALK1多肽,包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK4异聚体复合体可另包含本文所述ALK5多肽,包括例如与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK4异聚体复合体可另包含本文所述ALK6多肽,包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK4异聚体复合体可另包含本文所述ALK7多肽,包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK4异聚体复合体可另包含本文所述ALK7多肽,包括例如包含与选自SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。

[0021] 在一些实施方案中,本公开内容提供包含至少两种不同的I型TGF-β超家族受体多肽的异聚体多肽复合体,其中I型TGF-β超家族受体多肽的至少一种来源于ALK5受体。例如,ALK5多肽可包含与本文公开的ALK5序列(例如30、31、87、88、139、141、179、180、423、424、471和472)有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列或基本上由其组成或由其组成。任选ALK5多肽可包含与a)始于SEQ ID NO:30或87的氨基酸25-36的任一个(例如氨基酸残基25、26、27、28、29、30、31、32、33、34、35和36),和b)SEQ ID NO:30或87的氨基酸106-126的任一个(例如氨基酸残基106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列或基本上由其组成或由其组成的多肽。任选本公开内容的ALK5多肽可以是另包含对ALK5是异源的一个或多个部分(结构域)的融合蛋白。例如,ALK5多肽可与包含多聚化结构域的异源多肽融合,任选具有位于ALK5多肽和异源多肽之间的接头结构域。在一些实施方案中,本文所述多聚化结构域包

含相互作用对的一个组分。在一些实施方案中，包含ALK5多肽的异聚体复合体另包含至少一种不同的I型TGF-β超家族多肽。例如，ALK5异聚体复合体可另包含本文所述ALK2多肽，包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK5异聚体复合体可另包含本文所述ALK3多肽，包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK5异聚体复合体可另包含本文所述ALK4多肽，包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK5异聚体复合体可另包含本文所述ALK1多肽，包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK5异聚体复合体可另包含本文所述ALK6多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK5异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK5异聚体复合体可另包含本文所述ALK7多肽，包括例如包含与选自SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。

[0022] 在一些实施方案中，本公开内容提供包含至少两种不同的I型TGF-β超家族受体多肽的异聚体多肽复合体，其中I型TGF-β超家族受体多肽的至少一种来源于ALK6受体。例如，ALK6多肽可包含与本文公开的ALK6序列(例如34、35、91、92、142、144、181、182、425、426、473和474)有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选ALK6多肽可包含与a)始于SEQ ID NO:34的氨基酸14-32的任一个(例如氨基酸残基14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31和32)，和b)止于SEQ ID NO:34的氨基酸102-126的任一个(例如氨基酸残基102、103、104、105、106、107、108、109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125和126)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选ALK6多肽可包含与a)始于SEQ ID NO:91的氨基酸26-62的任一个(例如氨基酸残基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和62)，和b)止于SEQ ID NO:91的氨基酸132-156的任一个(例如氨基酸残基132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148、149、150、151、152、153、154、155和156)的多肽有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。任选公开内容的ALK6多肽本可以是另包含对ALK6是异

源的一个或多个部分(结构域)的融合蛋白。例如,ALK6多肽可与包含多聚化结构域的异源多肽融合,任选具有位于ALK6多肽和异源多肽之间的接头结构域。在一些实施方案中,本文所述多聚化结构域包含相互作用对的一个组分。在一些实施方案中,包含ALK6多肽的异聚体复合体另包含至少一种不同的I型TGF-β超家族多肽。例如,ALK6异聚体复合体可另包含本文所述ALK2多肽,包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK6异聚体复合体可另包含本文所述ALK3多肽,包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK6异聚体复合体可另包含本文所述ALK4多肽,包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK6异聚体复合体可另包含本文所述ALK5多肽,包括例如与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK6异聚体复合体可另包含本文所述ALK1多肽,包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK6异聚体复合体可另包含本文所述ALK7多肽,包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中,ALK6异聚体复合体可另包含本文所述ALK7多肽,包括例如包含与选自SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476的序列有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成的多肽。

[0023] 在一些实施方案中,本公开内容提供包含至少两种不同的I型TGF-β超家族受体多肽的异聚体多肽复合体,其中I型TGF-β超家族受体多肽的至少一种来源于ALK7受体。例如,ALK7多肽可包含与本文公开的ALK7序列(例如38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476)有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列或基本上由其组成或由其组成。任选ALK7多肽可包含与始于SEQ ID NO:38的氨基酸21-28的任一个(例如氨基酸21、22、23、24、25、26、27或28)和止于SEQ ID NO:38的氨基酸92-113的任一个(例如SEQ ID NO:38的氨基酸92、93、94、95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112或113)的多肽有至少70%、80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列或基本上由其组成或由其组成。任选本公开内容的ALK7多肽可以是另包含对ALK7是异源的一个或多个部分(结构域)的融合蛋白。例如,ALK7多肽可与包含多聚化结构域的异源多肽融合,任选具有位于ALK7多肽和异源多肽之间的接头结构域。在一些实施方案中,本文所述多聚化结构域包含相互作用对的一个组分。在一些实施方案中,包含ALK7多肽的异聚体复合体另包含至少一

种不同的I型TGF-β超家族多肽。例如，ALK7异聚体复合体可另包含本文所述ALK2多肽，包括例如包含与选自SEQ ID NO:18、19、136、138、173、174、421、422、465和466的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK7异聚体复合体可另包含本文所述ALK3多肽，包括例如包含与选自SEQ ID NO:22、23、115、117、175、176、407、408、467和468的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK7异聚体复合体可另包含本文所述ALK4多肽，包括例如包含与选自SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK7异聚体复合体可另包含本文所述ALK5多肽，包括例如与选自SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK7异聚体复合体可另包含本文所述ALK6多肽，包括例如包含与选自SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。在一些实施方案中，ALK7异聚体复合体可另包含本文所述ALK1多肽，包括例如包含与选自SEQ ID NO:14、15、124、126、171、172、413、414、463和464的序列有至少70％、80％、85％、90％、95％、97％、98％、99％或100％同一性的氨基酸序列、基本由其组成或由其组成的多肽。

[0024] 在一些实施方案中，本文公开的TGF-β超家族I型和/或II型受体多肽包含选自以下的一个或多个修饰的氨基酸残基：糖基化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化氨基酸、与脂质部分缀合的氨基酸和与有机衍化剂缀合的氨基酸。在一些实施方案中，本文所述TGF-β超家族I型和/或II型多肽被糖基化，并具有可获自多肽在哺乳动物细胞(包括例如CHO细胞)中表达的糖基化模式。

[0025] 在某些方面，本公开内容提供编码本文所述任何TGF-β超家族I型和/或II型多肽的核酸。本文公开的核酸可与启动子有效连接用于表达，本公开内容进一步提供用这类重组多核苷酸转化的细胞。优选细胞是哺乳动物细胞，例如COS细胞或CHO细胞。

[0026] 在某些方面，本公开内容提供用于制备本文所述TGF-β超家族I型和/或II型多肽的任一种以及包含所述多肽的蛋白质复合体的方法。所述方法可包括使本文公开的任何核酸在合适的细胞(例如CHO细胞或COS细胞)中表达。所述方法可包括：a)将细胞培养在适于本文所述TGF-β超家族I型或II型多肽表达的条件下，其中所述细胞用I型或II型多肽表达构建体转化；和b)回收所表达的I型或II型多肽。可采用从细胞培养物中获得蛋白质的任何众所周知的技术，回收作为粗产物、部分纯化或高度纯化的部分的本文所述TGF-β超家族I型和/或II型多肽以及所述多肽的蛋白质复合体。

[0027] 在某些方面，本公开内容提供用于制备本文公开的任何异多聚体复合体的方法。所述方法可包括使本文公开的任何核酸在合适的细胞(例如CHO细胞或COS细胞)中表达。所述方法可包括：a)获得包含含有本文公开的TGF-β超家族I型受体多肽的编码序列的核酸和包含本文公开的TGF-β超家族II型受体多肽的编码序列的核酸的细胞；(b)在适于本文所述TGF-β超家族I型和II型多肽表达的条件下培养所述细胞；和c)回收包含所表达的这类I型

和II型多肽的异聚体复合体。采用从细胞培养物获得蛋白质的任何众所周知的技术，回收作为粗产物、部分纯化或高度纯化的部分的本文公开的异多聚体复合体。

[0028] 可将本文描述的任何蛋白质复合体掺入药物制剂中。任选所述药物制剂相对于其它多肽组分是至少80％、85％、90％、95％、97％、98％或99％纯的。本文公开的任选药物制剂可包含一种或多种其它的活性剂。在一些实施方案中，本公开内容的异多聚体包含小于10％、9％、8％、7％、5％、4％、3％、2％或小于1％的I型受体多肽同多聚体。在一些实施方案中，本公开内容的异多聚体包含小于10％、9％、8％、7％、5％、4％、3％、2％或小于1％的II型受体多肽同多聚体。在一些实施方案中，本公开内容的异多聚体包含小于10％、9％、8％、7％、5％、4％、3％、2％或小于1％的I型受体多肽同多聚体和小于10％、9％、8％、7％、5％、4％、3％、2％或小于1％的II型受体多肽同多聚体。

[0029] 本公开内容进一步提供用于治疗或预防与例如受TGF-β超家族的一种或多种配体影响的肌肉、骨、脂肪、红细胞和其它组织有关的各种疾病和病症的方法和异多聚体复合体。所述疾病和病症包括但不限于与肌肉丢失或肌肉生长不足有关的病症(例如肌肉萎缩；肌营养不良，包括杜兴型肌营养不良、贝克肌营养不良和面肩胛肱骨肌营养不良；肌萎缩性侧索硬化和恶病质)和与不良体重增加有关的病症(例如肥胖症、2型糖尿病或非胰岛素依赖型糖尿病(NIDDM)、心血管疾病、高血压、骨关节炎、中风、呼吸问题和胆囊疾病)。在一些实施方案中，本文公开的异多聚体复合体可用于降低体脂肪含量或降低有需要的受试者的体脂肪含量增加的速率。在一些实施方案中，本文公开的异多聚体复合体可用于降低患者的胆固醇和/或甘油三酯水平。

[0030] 附图简述

[0031] 图1A和1B显示包含I型受体和II型受体多肽的异聚体蛋白质复合体的2个图解实例。图1A表示包含一个I型受体融合多肽和一个II型受体融合多肽的异二聚体蛋白质复合体，它可通过各多肽链内所含的多聚化结构域共价或非共价装配。两个装配的多聚化结构域构成相互作用对，它可以是受引导的或不受引导的。图2A表示包含与图1A一样的2个异二聚体复合体的杂四聚体蛋白质复合体。可预期更高阶的复合体。

[0032] 图2显示包含I型受体多肽(标示为“I”)(例如与来自人或其它物种例如本文所述其它物种的ALK1、ALK2、ALK3、ALK4、ALK5、ALK6或ALK7蛋白的胞外域有至少80％、85％、90％、95％、97％、98％、99％或100％同一性的多肽，例如SEQ ID NO:14、15、124、126、171、172、413、414、463、464、18、19、136、138、173、174、421、422、465、466、22、23、115、117、175、176、407、408、467、468、26、27、83、84、104、106、177、178、403、404、469、470、30、31、87、88、139、141、179、180、423、424、471、472、34、35、91、92、142、144、181、182、425、426、473、474、38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476)和II型受体多肽(标示为“II”)(例如与来自人或其它物种例如本文所述其它物种的ActRIIA、ActRIIB、MISRII、BMPRII或TGFBRII蛋白的胞外域有至少80％、85％、90％、95％、97％、98％、99％或100％同一性的多肽，例如9、10、11、118、120、151、152、409、410、451、452、1、2、3、4、5、6、100、102、153、154、401、402、453、454、46、47、71、72、121、123、155、156、411、412、455、456、50、51、75、76、79、80、133、135、161、162、419、420、457、458、42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462)的异聚体蛋白质复合体的图解实例。在说明性实施方案中，I型受体多肽是包含相互作用对的第一成员(“C”)的融合多

肽的一部分，II型受体多肽是包含相互作用对的第二成员(“D”)的融合多肽的一部分。在每个融合多肽中，接头可位于I型或II型受体多肽和相互作用对的相应成员之间。相互作用对的第一或第二成员(C,D)可以是受引导的(guided)(不对称)对，意味着所述对的成员优先彼此缔合而非自身缔合，或相互作用对可为不受引导的，意味着所述对的成员可彼此缔合或自身缔合而无实质性偏好且可具有相同或不同的氨基酸序列。传统的Fc融合蛋白和抗体是不受引导相互作用对的实例，而多种工程改造的Fc结构域被设计成受引导(不对称)相互作用对[例如，Spiess等(2015)Molecular Immunology67(2A):95-106]。

[0033] 图3显示根据多个ActRIIB和ActRIIA晶体结构的复合分析推导的具有直接接触用框标明的配体的残基的人ActRIIA(SEQ ID NO:500)和人ActRIIB(SEQ ID NO:2)的胞外域比对。

[0034] 图4显示无其胞内域的各种脊椎动物ActRIIB前体蛋白(分别为SEQ ID NO:501、502、503、504、505和506)、无其胞内域的人ActRIIA前体蛋白(SEQ ID NO:507)和共有ActRII前体蛋白(SEQ ID NO:508)的多个序列比对。

[0035] 图5显示应用Clustal2.1对来自人IgG同种型的Fc结构域的多个序列比对。铰链区用点下划线标示。双下划线表示IgG1 Fc中经工程改造以促进不对称链配对的位置和相对于其它同种型IgG2、IgG3和IgG4的相应位置的实例。

[0036] 图6显示ActRIIB-Fc:ALK4-Fc异二聚体蛋白质复合体与ActRIIB-Fc同二聚体和ALK4-Fc同二聚体相比的配体结合数据。对于各个蛋白质复合体，配体通过k_{off}(与配体信号转导抑制良好关联的动力学常数)排序，并以结合亲和力降序排列列出(结合最紧密的配体列在最前面)。在左边，黄色、红色、绿色和蓝色线表示解离速率常数量值。实心黑线表示其与异二聚体的结合与同二聚体相比提高或未改变的配体，而红色短划线表示与同二聚体相比结合大大减弱。如所示，ActRIIB-Fc:ALK4-Fc异二聚体显示与任一种同二聚体相比与激活素B的结合增强，与用ActRIIB-Fc同二聚体观察到的一样保持与激活素A、GDF8和GDF11牢固结合，且显示与BMP9、BMP10和GDF3的结合大大减弱。像ActRIIB-Fc同二聚体一样，异二聚体保持与BMP6的中等水平结合。

[0037] 图7显示ActRIIB-Fc:ALK3-Fc异二聚体蛋白质复合体与ActRIIB-Fc同二聚体和ALK3-Fc同二聚体相比的配体结合数据。格式与图6相同。如所示，ActRIIB-Fc:ALK3-Fc异二聚体以异常高的亲和力结合BMP2和BMP4，并且显示与任一种同二聚体相比与BMP5、BMP6、BMP7、GDF5、GDF6和GDF7的结合增强。与ActRIIB同二聚体相比，ActRIIB-Fc:ALK3-Fc异二聚体显示与激活素A、激活素B、BMP10、GDF8和GDF11的结合减弱，并且还区分这些配体至较大程度，尤其激活素A和激活素B之间。另外，对于ActRIIB-Fc:ALK3-Fc异二聚体，ActRIIB-Fc同二聚体以高亲和力结合BMP9和GDF3的能力不存在。

[0038] 图8显示ActRIIB-Fc:ALK7-Fc异二聚体蛋白质复合体与ActRIIB-Fc同二聚体和ALK7-Fc同二聚体相比的配体结合数据。格式与图6相同。如所示，与ActRIIB-Fc同二聚体牢固结合的4-5种配体(激活素A、BMP10、GDF8和GDF11)显示与ActRIIB-Fc:ALK7-Fc异二聚体的结合减弱，例外的是保持与异二聚体紧密结合的激活素B。另外，与ActRIIB-Fc同二聚体中等结合的3种配体(GDF3、BMP6和尤其BMP9)显示与ActRIIB-Fc:ALK7-Fc异二聚体的结合减弱。相比之下，BMP5以中等强度结合ActRIIB-Fc:ALK7异二聚体，尽管仅与ActRIIB-Fc同二聚体弱结合。无受测配体与ALK7-Fc同二聚体结合。

[0039] 图9显示ActRIIB-Fc:ALK2-Fc异二聚体蛋白质复合体与ActRIIB-Fc同二聚体和ALK2-Fc同二聚体相比的配体结合数据。格式与图6相同。如所示,ActRIIB-Fc:ALK2-Fc异二聚体显示与激活素B优先地牢固结合,因此类似于ActRIIB-Fc:ALK7-Fc异二聚体(图8)。然而,ActRIIB-Fc:ALK2-Fc异二聚体通过保持ActRIIB-Fc同二聚体特有的与BMP9紧密结合,而部分不同于ActRIIB-Fc:ALK7-Fc。无受测配体与ALK2-Fc同二聚体结合。

[0040] 图10显示ActRIIA-Fc:ALK4-Fc异二聚体蛋白质复合体与ActRIIA-Fc同二聚体和ALK4-Fc同二聚体相比的配体结合数据。格式与图6相同。如所示,ActRIIA-Fc:ALK4-Fc异二聚体显示与ActRIIA-Fc同二聚体相比,与激活素A的结合增强,尤其与激活素AC的结合增强,同时保持与激活素AB和GDF11牢固结合。另外,具有对ActRIIA-Fc同二聚体有最高亲和力的配体激活素B显示对ActRIIA-Fc:ALK4-Fc异二聚体的亲和力降低(尽管仍在高亲和力范围内)。ActRIIA-Fc:ALK4-Fc异二聚体还显示与ActRIIA-Fc同二聚体相比与BMP10的结合显著减弱。

[0041] 图11显示与ActRIIB-Fc同二聚体和ALK1-Fc同二聚体相比BMPRII-Fc:ALK1-Fc异二聚体蛋白质复合体的配体结合数据。格式与图6相同。如所示,BMPRII-Fc:ALK1-Fc异二聚体大多保持ALK1-Fc同二聚体特有的与BMP9和BMP10牢固结合;然而,异二聚体显示用同二聚体不存在的针对BMP10优于BMP9的适度选择性。此外与ALK1-Fc同二聚体不一样,BMPRII-Fc:ALK1-Fc异二聚体与BMP15结合,尽管解离速率约为BMPRII-Fc同二聚体的10倍。

[0042] 图12显示BMPRII-Fc:ALK3-Fc异二聚体蛋白质复合体与BMPRII-Fc同二聚体和ALK3-Fc同二聚体相比的配体结合数据。格式与图6相同。如所示,BMPRII-Fc:ALK3-Fc异二聚体比ALK3-Fc同二聚体牢得多地与BMP6结合,反映了解离速率接近1/10。由于其与BMP2和BMP4的结合大多未改变,BMPRII-Fc:ALK3异二聚体因此可被视为BMP2、BMP4和BMP6的联合抑制剂(joint inhibitor)。这种结合特征与ALK3-Fc同二聚体的截然不同,其与BMP4和BMP2异常牢固地结合将其鉴定为与以中等水平结合的4种配体(包括BMP6)相比对该配体对有高选择性。

[0043] 图13显示BMPRII-Fc:ALK4-Fc异二聚体蛋白质复合体与BMPRII-Fc同二聚体和ALK4-Fc同二聚体相比的配体结合数据。格式与图6相同。BMPRII-Fc:ALK4-Fc异二聚体因以高或中等强度结合几种激活素配体而不同于两种同二聚体,且因仅弱结合BMP15而不同于BMPRII-Fc同二聚体。最值得注意的是,BMPRII-Fc:ALK4-Fc异二聚体牢固且以高选择性与异二聚体配体激活素AB结合。

[0044] 图14显示两种不同TGFβRII-Fc:ALK5-Fc异二聚体蛋白质复合体与TGFβRII-Fc同二聚体和ALK5-Fc同二聚体相比的配体结合数据。格式与图6相同。如所示,TGFβRII-Fc:ALK5-Fc异二聚体因其对TGFβ2的高选择性同时仍保持对TGFβ1和TGFβ3相当多的亲和力而显著不同于TGFβRII-Fc同二聚体。掺入TGFβRII结合的TGFβ2的长同种型的异二聚体比其短同种型对应物更牢和更多选择性。无受测配体与ALK5-Fc同二聚体结合。

[0045] 图15A-15D显示包含I型受体多肽(标示为“I”)(例如与来自人或其它物种例如本文所述其它物种的ALK1、ALK2、ALK3、ALK4、ALK5、ALK6或ALK7蛋白的胞外域有至少80%、85%、90%、95%、97%、98%、99%或100%同一性的多肽,例如SEQ ID NO:14、15、124、126、171、172、413、414、463、464、18、19、136、138、173、174、421、422、465、466、22、23、115、117、175、176、407、408、467、468、26、27、83、84、104、106、177、178、403、404、469、470、30、31、87、

88、139、141、179、180、423、424、471、472、34、35、91、92、142、144、181、182、425、426、473、474、38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476)和II型受体多肽(标示为“II”)(例如与来自人或其它物种例如本文所述其它物种的ActRIIA、ActRIIB、MISRII、BMPRII或TGFBRII蛋白的胞外域有至少80％、85％、90％、95％、97％、98％、99％或100％同一性的多肽,例如9、10、11、118、120、151、152、409、410、451、452、1、2、3、4、5、6、100、102、153、154、401、402、453、454、46、47、71、72、121、123、155、156、411、412、455、456、50、51、75、76、79、80、133、135、161、162、419、420、457、458、42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462)的异聚体蛋白质复合体的图解实例。在说明性实施方案中,I型受体多肽是包含相互作用对的第一成员(“C_1”)的融合多肽的一部分,II型受体多肽是包含相互作用对的第二成员(“C_2”)的融合多肽的一部分。合适的相互作用对包括例如重链和/或轻链免疫球蛋白相互作用对、其截短物和变体例如本文描述的那些[例如,Spiess等(2015)Molecular Immunology67(2A):95-106]。在每个融合多肽中,接头可位于I型受体多肽或II型受体多肽和相互作用对的相应成员之间。相互作用对的第一或第二成员可以是不受引导的,这意味着所述对的成员可彼此缔合或自身缔合而无实质性偏好,且它们可具有相同或不同的氨基酸序列。参见图15A。或者,相互作用对可以是受引导(不对称)对,这意味着所述对的成员优先彼此缔合而非自身缔合。参见图15B。可预期更高阶的复合体。参见图15C和15D。

[0046] 图16A-16G显示包含两种I型受体多肽(标示为“I”)(例如与来自人或其它物种例如本文所述其它物种的ALK1、ALK2、ALK3、ALK4、ALK5、ALK6或ALK7蛋白的胞外域有至少80％、85％、90％、95％、97％、98％、99％或100％同一性的多肽,例如SEQ ID NO:14、15、124、126、171、172、413、414、463、464、18、19、136、138、173、174、421、422、465、466、22、23、115、117、175、176、407、408、467、468、26、27、83、84、104、106、177、178、403、404、469、470、30、31、87、88、139、141、179、180、423、424、471、472、34、35、91、92、142、144、181、182、425、426、473、474、38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476)和两种II型受体多肽(标示为“II”)(例如与来自人或其它物种例如本文所述其它物种的ActRIIA、ActRIIB、MISRII、BMPRII或TGFBRII蛋白的胞外域有至少80％、85％、90％、95％、97％、98％、99％或100％同一性的多肽,例如9、10、11、118、120、151、152、409、410、451、452、1、2、3、4、5、6、100、102、153、154、401、402、453、454、46、47、71、72、121、123、155、156、411、412、455、456、50、51、75、76、79、80、133、135、161、162、419、420、457、458、42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462)的异聚体蛋白质复合体的图解实例。

[0047] 在说明性实施方案16A中,第一I型受体多肽(从左到右)是包含相互作用对的第一成员(“C_1”)和另包含其它相互作用对的第一成员(“A_1”)的融合多肽的一部分;第二I型受体多肽是包含相互作用对的第二成员(“C_2”)和另包含相互作用对的第一成员(“A_2”)的融合多肽的一部分。第一II型受体多肽(从左到右)是包含相互作用对的第二成员(“B_1”)的融合多肽的一部分;第二II型受体多肽是包含相互作用对的第二成员(“B_2”)的融合多肽的一部分。A_1和A_2可相同或不同;B_1和B_2可相同或不同,且C_1和C_2可相同或不同。在每个融合多肽中,接头可位于I型受体多肽或II型受体多肽和相互作用对的相应成员之间以及相互作用对之间。图9A是不受引导相互作用对的缔合的实例,这意味着所述对的成员可彼此缔合或自身

缔合而无实质性偏好且可具有相同或不同的氨基酸序列。

[0048] 在说明性实施方案16B中，第一II型受体多肽(从左到右)是包含相互作用对的第一成员(“C_1”)和另包含其它相互作用对的第一成员(“A_1”)的融合多肽的一部分；第二II型受体ActRIIB多肽是包含相互作用对的第二成员(“B_2”)的融合多肽的一部分。第一I型受体多肽(从左到右)是包含相互作用对的第二成员(“B_1”)的融合多肽的一部分；第二I型受体多肽是包含相互作用对的第二成员(“C_2”)和另包含相互作用对的第一成员(“A_2”)的融合多肽的一部分。在每个融合多肽中，接头可位于I型受体或II型受体多肽和相互作用对的相应成员之间以及相互作用对之间。图16B是受引导(不对称)相互作用对缔合的实例，这意味着所述对的成员优先彼此缔合而非自身缔合。

[0049] 合适的相互作用对包括例如本文描述的重链和/或轻链免疫球蛋白相互作用对、其截短物和变体[例如，Spiess等(2015)Molecular Immunology67(2A)：95-106]。可预期更高阶的复合体。参见图16C-16F。采用类似方法，特别是使用轻链和/或重链免疫球蛋白、其截短物或变体的方法，可将相互作用对用于产生类似于抗体Fab和$F(ab')_2$复合体的异二聚体[例如，Spiess等(2015)Molecular Immunology67(2A)：95-106]。参见图16G。

[0050] 图17A和17B显示包含I型受体多肽(标示为“I”)(例如与来自人或其它物种例如本文所述其它物种的ALK1、ALK2、ALK3、ALK4、ALK5、ALK6或ALK7蛋白的胞外域有至少80％、85％、90％、95％、97％、98％、99％或100％同一性的多肽，例如SEQ ID NO:14、15、124、126、171、172、413、414、463、464、18、19、136、138、173、174、421、422、465、466、22、23、115、117、175、176、407、408、467、468、26、27、83、84、104、106、177、178、403、404、469、470、30、31、87、88、139、141、179、180、423、424、471、472、34、35、91、92、142、144、181、182、425、426、473、474、38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476)和II型受体多肽(标示为“II”)(例如与来自人或其它物种例如本文所述其它物种的ActRIIA、ActRIIB、MISRII、BMPRII或TGFBRII蛋白的胞外域有至少80％、85％、90％、95％、97％、98％、99％或100％同一性的多肽，例如9、10、11、118、120、151、152、409、410、451、452、1、2、3、4、5、6、100、102、153、154、401、402、453、454、46、47、71、72、121、123、155、156、411、412、455、456、50、51、75、76、79、80、133、135、161、162、419、420、457、458、42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462)和抗体的配体结合域(例如来源于与一种或多种TGF-β超家族配体结合的抗体的配体结合结构域)的异聚体蛋白质复合体的图解实例。在说明性实施方案中，I型受体多肽是包含相互作用对的第一成员(“C_1”)和另包含其它相互作用对的第一成员(“A_1”)的融合多肽的一部分。II型受体多肽是包含相互作用对的第二成员(“B_1”)的融合多肽的一部分。可变重链(V_H)多肽是包含相互作用对的第二成员(“C_2”)和另包含相互作用对的第一成员(“A_2”)的融合多肽的一部分。可变重链(V_L)多肽是包含相互作用对的第二成员(“B_2”)的融合多肽的一部分。在每个融合多肽中，接头可位于I型受体或II型受体多肽和相互作用对的相应成员之间、相互作用对之间和V_H和V_L多肽和相互作用对的成员之间。A_1和A_2可相同或不同；B_1和B_2可相同或不同，且C_1和C_2可相同或不同。合适的相互作用对包括例如本文描述的恒定重链和/或轻链免疫球蛋白相互作用对、其截短物和变体[例如，Spiess等(2015)Molecular Immunology67(2A)：95-106]。图17A是受引导(不对称)相互作用对缔合的实例，这意味着所述对的成员优先彼此缔合而非自身缔合。图17B是不受引导相互作用对的缔合的实例，这意味着所述对的成员可彼

此缔合或自身缔合而无实质性偏好且可具有相同或不同的氨基酸序列。

[0051] 图18显示图解实例I型受体:II型受体单阱(single-trap)多肽。I型受体:II型受体单阱多肽可含有具有相同或不同序列的多个I型受体结构域(例如1、2、3、4、5、6、7、9、10个或更多个结构域)和具有相同或不同序列的多个II型受体结构域(例如1、2、3、4、5、6、7、9、10个或更多个结构域)。这些I型受体和II型受体结构域可以任何顺序排列,并且在I型1受体和II型受体结构域的一个或多个之间可包含一个或多个接头结构域位置。所述配体阱可用作治疗或预防本文所述疾病或病症的治疗剂。

[0052] 图19A-19D显示包含至少一个I型受体:II型受体单链阱(single chain trap)多肽的多聚体蛋白质复合体的图解实例。在说明性实施方案19A和19B中,第一I型受体:II型受体单链阱多肽(从左到右)是包含相互作用对的第一成员(“C_1”)的融合多肽的一部分;第二I型受体:II型受体单链阱多肽是包含相互作用对的第二成员(“C_2”)的融合多肽的一部分。C_1和C_2可相同或不同。第一和第二I型受体:II型受体单链阱多肽可相同或不同。在每个融合多肽中,接头可位于I型受体:II型受体单链阱多肽和相互作用对的相应成员之间。合适的相互作用对包括例如本文描述的重链和/或轻链免疫球蛋白相互作用对、其截短物和变体[例如,Spiess等(2015)Molecular Immunology67(2A):95-106]。图19A是不受引导相互作用对的缔合的实例,这意味着所述对的成员可彼此缔合或自身缔合而无实质性偏好且可具有相同或不同的氨基酸序列。图129是受引导(不对称)相互作用对缔合的实例,这意味着所述对的成员优先彼此缔合而非自身缔合。可预期更高阶的复合体。另外,这类I型受体:II型受体单链阱多肽同样可与一种或多种I型受体多肽和/或一种或多种II型受体多肽共价或非共价缔合。参见图19C。同样,这类I型受体:II型受体单链阱多肽同样可与共价或非共价缔合抗体的一个或多个配体结合域(例如与一种或多种I型受体:II型受体异多聚体结合配体结合的抗体的配体结合结构域)。参见图19D。

[0053] 发明详述

[0054] 1.概述

[0055] 一方面,本公开内容涉及包含TGFβ超家族I型受体多肽的胞外域和TGFβ超家族II型受体多肽的胞外域的异多聚体复合体、包含至少两种不同的TGFβ超家族I型受体多肽的胞外域的异多聚体复合体、包含至少两种不同的TGFβ超家族II型受体多肽的胞外域的异多聚体复合体、制备所述异多聚体复合体的方法及其用途。如本文所述,在一些实施方案中,异多聚体复合体可包含选自以下的TGFβ超家族I型受体多肽的胞外域:ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7。同样,在一些实施方案中,这些异多聚体复合体可包含选自以下的TGFβ超家族II型受体多肽的胞外域:ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII。在某些优选的实施方案中,相对于同多聚体复合体的相应样本,本公开内容的异多聚体复合体的TGFβ超家族配体结合特异性/概况改变(例如ActRIIB:ALK4异二聚体与ActRIIB:ActRIIB同二聚体复合体或ALK4:ALK4同二聚体复合体相比较)。

[0056] TGF-β超家族由30种以上的分泌的因子组成,包括TGF-β、激活素、nodal、骨形态发生蛋白(BMP)、生长分化因子(GDF)和抗缪勒氏管激素(anti-Mullerian hormone,AMH)。参见例如Weiss等(2013)Developmental Biology,2(1):47-63。存在于脊椎动物和无脊椎动物两者中的超家族的成员,在动物生命发育的最早期在不同的组织和功能中无所不在地表达。实际上,TGF-β超家族蛋白质是干细胞自我更新、原肠胚形成、分化、器官形态发生和成

体组织稳态的关键介质。与这种普遍存在的活性一致，异常的TGF-β超家族信号转导与包括例如自身免疫性疾病、心血管疾病、纤维变性疾病和癌症在内的多种人类病理有关。

[0057] TGF-β超家族的配体具有相同的二聚结构，其中一个单体的中心3-1/2转角螺旋紧靠由其它单体的β-折叠链形成的凹面上堆积。TGF-β家族成员的大多数被分子间二硫键进一步稳定。该二硫键跨越由两个其它二硫键形成的环，产生所称的‘半胱氨酸结(cysteine knot)’基序。参见例如Lin等(2006)Reproduction132:179-190和Hinck等(2012)FEBS Letters 586:1860-1870。

[0058] TGF-β超家族信号转导通过I型和II型丝氨酸/苏氨酸激酶受体的异聚体复合体介导，这在配体刺激时使下游SMAD蛋白(例如SMAD蛋白1、2、3、5和8)磷酸化并将其激活。参见例如Massagué(2000)Nat.Rev.Mol.CellBiol.1:169-178。这些I型和II型受体是跨膜蛋白，由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶特异性的胞质结构域组成。一般而言，I型受体介导胞内信号转导，而II型受体是结合TGF-β超家族配体所必需的。I型和II受体在配体结合后形成稳定的复合体，导致I型受体被II型受体磷酸化。

[0059] 可根据它们结合的I型受体和它们激活的SMAD蛋白，将TGF-β家族分成两个系统发育分支。一个是更近进化支，这包括例如TGF-β、激活素、GDF8、GDF9、GDF11、BMP3和nodal，它通过激活Smad2和3的I型受体传送信号[Hinck(2012)FEBSLetters586:1860-1870]。另一支包含较远缘的超家族蛋白质，包括例如BMP2、BMP4、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF1、GDF5、GDF6和GDF7，其通过Smad1、5和8传送信号。

[0060] TGF-β同种型是TGF-β超家族的创立成员，其中在哺乳动物中有3种已知的同种型，名为TGF-β1、TGF-β2和TGF-β3。有生物活性的成熟TGF-β配体起同二聚体的作用，主要通过I型受体ALK5传送信号，但还发现在内皮细胞中另通过ALK1传送信号。参见例如Goumans等(2003)Mol Cell12(4):817-828。TGF-β1是最丰富和遍在表达的同种型。已知TGF-β1在伤口愈合中具有重要作用，且表达组成型活性TGF-β1转基因的小鼠发生纤维变性。参见例如Clouthier等(1997)JClin.Invest.100(11):2697-2713。TGF-β1还参与T细胞活化和T调节细胞维持。参见例如Li等(2006)Immunity25(3):455-471。TGF-β2表达最早描述于人成胶质细胞瘤细胞中，并出现在胚胎神经系统的神经元和星形胶质细胞中。已知TGF-β2抑制T淋巴细胞的白介素-2依赖性生长。TGF-β3最初从人横纹肌肉瘤细胞系中分离，从此以后一直存在于肺腺癌和肾癌细胞系。已知TGF-β3对腭和肺形态发生是重要的。参见例如Kubiczkova等(2012)Journal of Translational Medicine10:183。

[0061] 激活素是TGF-β超家族的成员，且最初发现作为促卵泡激素分泌的调节物，但随后表征了各种生殖和非生殖作用。有3种主要的激活素形式(A、B和AB)，其是两个密切相关的β亚基的同/异二聚体(分别为$\beta_A\beta_A$、$\beta_B\beta_B$和$\beta_A\beta_B$)。人基因组还编码主要在肝中表达的激活素C和激活素E，且含有β_C或β_E的异二聚体形式也是已知的。在TGF-β超家族中，激活素是独特的多功能因子，可刺激卵巢和胎盘细胞的激素产生、支持神经元细胞存活、根据细胞类型正面或负面影响细胞周期进程，并且至少在两栖动物胚胎中诱导中胚层分化DePaolo等(1991)Proc Soc Ep Biol Med.198:500-512;Dyson等(1997)Curr Biol.7:81-84;以及Woodruff(1998)Biochem Pharmacol.55:953-963。在若干组织中，激活素信号转导被其相关的异二聚体抑制素拮抗。例如在来自垂体的促卵泡激素(FSH)分泌的调节中，激活素促进FSH合成

和分泌，同时抑制素降低FSH合成和分泌。可调节激活素生物活性和/或与激活素结合的其它蛋白质包括促滤泡素抑制素(FS)、促滤泡素抑制素相关蛋白(FSRP，亦称为FLRG或FSTL3)和α_2-巨球蛋白。

[0062] 如本文所述，与“激活素A”结合的作用剂是与β_A亚基特异性结合的作用剂，不论在分离的β_A亚基还是作为二聚体复合体(例如$\beta_A\beta_A$同二聚体或$\beta_A\beta_B$异二聚体)的情况下。在异二聚体复合体(例如$\beta_A\beta_B$异二聚体)的情况下，与“激活素A”结合的作用剂对β_A亚基内存在的表位有特异性，但不与复合体的非β_A亚基(例如复合体的β_B亚基)内存在的表位结合。同样，本文公开的拮抗(抑制)“激活素A”的作用剂是抑制被β_A亚基介导的一种或多种活性的作用剂，不论在分离的β_A亚基还是作为二聚体复合体(例如$\beta_A\beta_A$同二聚体或$\beta_A\beta_B$异二聚体)的情况下。在$\beta_A\beta_B$异二聚体的情况下，抑制“激活素A”的作用剂是特异性抑制β_A亚基的一种或多种活性，但不抑制复合体的非β_A亚基(例如复合体的β_B亚基)的活性的作用剂。该原则同样适用于结合和/或抑制“激活素B”、“激活素C”和“激活素E”的作用剂。本文公开拮抗“激活素AB”、“激活素AC”、“激活素AE”、“激活素BC”或“激活素BE”的作用剂是抑制作为被β_A亚基介导的一种或多种活性和作为被β_B亚基介导的一种或多种活性的作用剂。相同原则适用于结合和/或抑制“激活素AC”、“激活素AE”、“激活素BC”或“激活素BE”的作用剂。

[0063] Nodal蛋白在中胚层和内胚层诱导和形成以及在早期胚胎发生的中轴结构(例如心脏和胃)随后的组构中起作用。已表明发育中的脊椎动物胚胎的背部组织主要促进脊索和脊索前板的中轴结构，同时它募集周围细胞以形成非中轴胚胎结构。Nodal似乎通过I型和II型受体和称为SMAD蛋白的胞内效应子两者传送信号。研究支持这样的构想，即ActRIIA和ActRIIB起针对nodal的II型受体的作用。参见例如Sakuma等(2002)Genes Cells.2002,7:401-12。曾指出Nodal配体与其辅因子(例如Cripto或Cryptic)相互作用以激活激活素I型和II型受体，这使SMAD2磷酸化。Nodal蛋白涉及对早期脊椎动物胚胎至关重要的许多事件，包括中胚层形成、前部图式形成(anterior patterning)和左-右轴特化。实验证据表明nodal信号转导刺激pAR3-Lux(一种之前表明对激活素和TGF-β特异性起反应的一种萤光素酶报道分子)。然而，nodal不能够诱导pTlx2-Lux(一种对骨形态发生蛋白起特异性反应的报道分子)。最新结果提供直接的生物化学证据，即nodal信号转导受SMAD2和SMAD3介导，SMAD2和SMAD3还介导由TGF-β和激活素引起的信号转导。更多的证据表明胞外蛋白质Cripto或Cryptic是nodal信号转导所必需的，使之与激活素或TGF-β信号转导截然不同。

[0064] BMP和GDF一起形成共有TGF-β超家族的特征性折叠的半胱氨酸结细胞因子的家族。参见例如Rider等(2010)BiochemJ.,429(1):1-12。该家族包括例如BMP2、BMP4、BMP6、BMP7、BMP2a、BMP3、BMP3b(亦称为GDF10)、BMP4、BMP5、BMP6、BMP7、BMP8、BMP8a、BMP8b、BMP9(亦称为GDF2)、BMP10、BMP11(亦称为GDF11)、BMP12(亦称为GDF7)、BMP13(亦称为GDF6)、BMP14(亦称为GDF5)、BMP15、GDF1、GDF3(亦称为VGR2)、GDF8(亦称为肌肉生长抑制素)、GDF9、GDF15和十五褶(decapentaplegic)。除诱导骨形成的能力(BMP因此得名)以外，BMP/GDF在各种组织发育中显示形态发生活性。BMP/GDF同二聚体和异二聚体与I型和II型受体二聚体的组合相互作用产生多种可能的信号转导复合体，导致两套竞争性SMAD转录因子之一的活化。BMP/GDF具有高度特异性和局部化功能。这些以多种方式受调节，包括BMP/GDF表达的发育限制和通过以高亲和力与细胞因子结合的几种特异性BMP拮抗剂蛋白的分泌。令人好奇的是，多种这些拮抗剂像TGF-β超家族配体。

[0065] 生长分化因子-8(GDF8)亦称为肌肉生长抑制素。GDF8是骨骼肌质量的负调节剂，并在发育和成熟骨骼肌中高度表达。转基因小鼠中GDF8无效突变的特征在于骨骼肌显著肥大和增生。参见例如McPherron等,Nature(1997)387:83-90。在牛中,惊人地在人中,骨骼肌质量的类似增加在GDF8的天然存在的突变中是明显的。参见例如Ashmore等(1974)Growth,38:501-507;Swatland和Kieffer,J.Anim.Sci.(1994)38:752-757;McPherron和Lee,Proc.Natl.Acad.Sci.USA(1997)94:12457-12461;Kambadur等,GenomeRes.(1997)7:910-915;以及Schuelke等(2004)N Engl J Med,350:2682-8。研究还显示,与人类HIV感染有关的肌肉消耗伴有GDF8蛋白表达增加。参见例如Gonzalez-Cadavid等,PNAS(1998)95:14938-43。另外,GDF8可调节肌肉特异性酶(例如肌酸激酶)的产生并调节成肌细胞增殖。参见例如国际专利申请公布号WOOO/43781)。GDF8前肽可与成熟的GDF8结构域二聚体非共价结合,使其生物活性钝化。参见例如Miyazono等(1988)J.Biol.Chem.,263:6407-6415;Wakefield等(1988)J.Biol.Chem.,263;7646-7654;以及Brown等(1990)Growth Factors,3:35-43。与GDF8或结构相关蛋白结合并抑制其生物活性的其它蛋白质包括促滤泡素抑制素,并可能促滤泡素抑制素相关蛋白。参见例如Gamer等(1999)Dev.Biol.,208:222-232。

[0066] GDF11,亦称为BMP11,是在小鼠发育期间在尾芽、肢芽、腭上颌弓和下颌弓及背根神经节表达的分泌蛋白。参见例如McPherron等(1999)Nat.Genet.,22:260-264;以及Nakashima等(1999)Mech.Dev.,80:185-189。GDF11在中胚层和神经组织两者的图式形成中起独特作用。参见例如Gamer等(1999)Dev Biol.,208:222-32。表明了GDF11在发育中的鸡肢中是软骨发生和肌生成的负调节剂。参见例如Gamer等(2001)Dev Biol.,229:407-20。GDF11在肌肉中的表达还表明其以与GDF8类似的方式在调节肌肉生长中的作用。另外,GDF11在脑中的表达表明GDF11还可具有涉及神经系统功能的活性。引人关注的是,发现GDF11在嗅上皮中抑制神经发生。参见例如Wu等(2003)Neuron.,37:197-207。因此,GDF11可在疾病例如肌肉疾病和神经变性疾病(例如肌萎缩性侧索硬化)的治疗中具有体外和体内应用。

[0067] 众所周知BMP7,亦称成骨蛋白-1(OP-1)诱导软骨和骨形成。另外,BMP7调节多种生理过程。例如,BMP7可能是负责上皮骨发生现象的骨诱导因子。还发现BMP7在钙调节和骨稳态中起作用。像激活素一样,BMP7与II型受体ActRIIA和ActRIIB结合。然而,BMP7和激活素将不同的I型受体募集到异聚体受体复合体。所观察到的主要BMP7I型受体是ALK2,而激活素专门与ALK4结合(ActRIIB)。BMP7和激活素诱导截然不同的生物应答,并刺激不同的SMAD途径。参见例如Macias-Silva等(1998)J Biol Chem.273:25628-36。

[0068] 抗缪勒氏管激素(AMH),亦称为缪勒氏管抑制物质(Mullerian-inhibiting substance,MIS),是TGF-β家族糖蛋白。已鉴定出一种AMH相关的II型受体,命名为AMHRII或者MISRII。AMH诱导人类男性胚胎中缪勒氏管的消退。AMH在生殖年龄女性中表达,并且不随生理期或妊娠波动,但因卵母细胞数量和质量两者降低而逐步减少,表明AMH可用作卵巢生理学的生物标志物。参见例如Zec等(2011)Biochemia Medica21(3):219-30。

[0069] 激活素受体样激酶-1(ALK1),ACVRL1基因的产物,或称为ACVRLK1,是其表达主要受限于内皮细胞的I型受体。参见例如OMIM条目601284。ALK1受TGF-β家族配体(例如BMP9和BMP10)结合的刺激,且ALK1信号转导在发育性和病理性血管形成两者的调节中至关重要。ALK1表达在早期小鼠发育中与血管发生和血管生成的部位重叠,ALK1敲除小鼠因严重的血

管异常在胚期第11.5天左右死亡(参见例如Cunha和Pietras(2011)Blood117(26):6999-7006)。ALK1表达还描述于其它细胞类型例如肝星状细胞和软骨细胞中。另外,发现ALK1连同激活素受体样激酶-2(ALK2)对间充质干细胞中的BMP9诱导的成骨信号转导是重要的。参见例如Cunha和Pietras(2011)Blood117(26):6999-7006。

[0070] ALK2,ACVR1基因的产物,另称为ActRIA或ACVRLK2,是已表明结合激活素和BMP的I型受体。ALK2对胚胎发生至关重要,因为ALK2敲除小鼠在原肠胚形成后不久死亡。参见例如Mishina等(1999)Dev Biol.213:314-326和OMIM条目102576。ALK2的组成型活性突变与进行性骨化性纤维发育不良(FOP)有关。FOP是一种引起纤维组织(包括肌肉、肌腱和韧带)自发骨化或当受损时的罕见遗传紊乱。在人类中,ALK2密码子206中精氨酸向组氨酸突变与FOP天然存在的突变有关。该突变在无配体结合时通过ALK2诱导BMP特异性信号转导。参见例如Fukuda等(2009)J Biol Chem.284(11):7149-7156和Kaplan等(2011)Ann N.Y.Acad Sci.1237:5-10。

[0071] 激活素受体样激酶-3(ALK3),BMPR1A基因的产物,亦称为ACVRLK3,是介导BMP家族的多种配体的作用的I型受体。与具有遍在组织表达的几种I型受体不一样,ALK3显示与更特化的功能性一致的受限表达模式。参见例如ten Dijke(1993)Oncogene,8:2879-2887和OMIM条目601299。ALK3一般被视为对BMP2、BMP4、BMP7和其它BMP家族成员的高亲和力受体。BMP2和BMP7是成骨细胞分化的有效刺激物,目前在临床上在脊柱融合和某些不愈合骨折中用于诱导骨形成。ALK3被视为在成骨细胞中介导BMP2和BMP4信号转导的关键受体。参见例如Lavery等(2008)J.Biol.Chem.283:20948-20958。纯合子ALK3敲除小鼠早在胚胎发生(大约第9.5天)时便死亡,然而,最近报导了在成骨细胞中携带条件性ALK3破坏的成年小鼠显示骨质增加,尽管新形成的骨显示结构破坏的证据。参见例如Kamiya(2008)J.Bone Miner.Res.,23:2007-2017;以及Kamiya(2008)Development135:3801-3811。这个结果与在临床应用中作为成骨剂的BMP2和BMP7(配体for ALK3)的效应惊人地不同。

[0072] 激活素受体样激酶-4(ALK4),ACVR1B基因的产物,亦称为ACVRLK4,是转导多种TGF-β家族配体(包括激活素、nodal和GDF)的信号转导的I型受体。ALK4突变与胰腺癌有关,并且显性负截短的ALK4同种型在人垂体肿瘤中高表达。参见例如Tsuchida等(2008)Endocrine Journal55(1):11-21和OMIM条目601300。

[0073] 激活素受体样激酶-5(ALK5),TGFBR1基因的产物,在大多数细胞类型中广泛表达。几种TGF-β超家族配体,包括TGF-β、激活素和GDF-8,通过ALK5传送信号,并激活下游Smad2和Smad3。小鼠ALK5缺陷型在卵黄囊和胎盘的血管发育中显示严重缺陷,缺乏循环红细胞,并在妊娠中期死亡。发现这些胚胎具有正常的造血潜力,但增殖增加,且内皮细胞不当迁移。因此,ALK5依赖性信号转导对血管生成是重要的,但对造血祖细胞的发育和功能性血细胞生成不重要。参见例如Larsson等(2001)The EMBO Journal,20(7):1663-1673和OMIM条目190181。在内皮细胞中,ALK5协同作用并与ALK1信号转导相反。ALK5抑制细胞迁移和增殖,值得注意的是ALK1的相反作用。参见例如Goumans等(2003)Mol Cell 12(4):817-828。另外,ALK5被认为负调节肌肉生长。发现肌肉中的ALK5敲减(一种肌营养不良小鼠模型)减少纤维变性并提高与肌肉生长相关的基因的表达。参见例如Kemaladewi等(2014)Mol Ther Nucleic Acids3,e156。

[0074] 激活素受体样激酶-6(ALK6)是BMPR1B基因的产物,其缺乏与人和小鼠两者的软骨

发育异常和四肢缺陷有关。参见例如Demirhan等(2005)J Med Genet.42:314-317。在小鼠中,ALK6在整个发育中的骨骼中广泛表达,并且是软骨发生所必需的。参见例如Yi等(2000)Development127:621-630和OMIM条目603248。

[0075] 激活素受体样激酶-7(ALK7)是ACVR1C基因的产物。ALK7失效小鼠是有活力、能育的,并显示无骨骼或肢畸形。GDF3通过ALK7的信号转导似乎在胰岛素敏感性和肥胖症中起作用。这得到Alk7失效小鼠显示脂肪蓄积和对饮食诱导的肥胖症的抗性降低的结果的支持。参见例如Andersson等(2008)PNAS105(20):7252-7256。在多种不同的癌细胞系中,ALK7介导的Nodal信号转导涉及肿瘤促进和肿瘤抑制作用两者。参见例如De Silva等(2012)Frontiers in Endocrinology3:59和OMIM条目608981。

[0076] 本文所用术语"ActRII"是指II型激活素受体的家族。该家族包括由ACVR2A基因编码的激活素受体IIA型(ActRIIA)和由ACVR2B基因编码的激活素受体II B型(ActRIIB)两者。ActRII受体是结合多种TGF-β超家族配体的TGF-β超家族II型受体,所述配体包括激活素、GDF8(肌肉生长抑制素)、GDF11和BMP亚型,值得注意的是BMP6和BMP7。ActRII受体涉及多种生物病症,包括肌肉和神经肌肉病症(例如肌营养不良、肌萎缩性侧索硬化(ALS)和肌肉萎缩)、不需要的骨/软骨生长、脂肪组织病症(例如肥胖症)、代谢紊乱(例如2型糖尿病)和神经变性病症。参见例如Tsuchida等(2008)Endocrine Journal55(1):11-21;Knopf等,U.S.8,252,900和OMIM条目102581和602730。

[0077] 由TGFBR2基因编码的转化生长因子β受体II(TGFBRII)是已知结合TGF-β配体并激活下游Smad2和Smad3效应子的II型受体。参见例如Hinck(2012)FEBS Letters586:1860-1870和OMIM条目190182。TGF-β通过TGFBRII的信号转导在T细胞增殖、T调节细胞维持和前软骨干细胞增殖中至关重要。参见例如Li等(2006)Immunity25(3):455-471和Cheng等,Int.J.Mol.Sci.2014,15,12665-12676。

[0078] 由BMPR2基因编码的骨形态发生蛋白受体II(BMPRII)是被认为结合某些BMP配体的II型受体。在某些情况下,与BMPRII的有效配体结合依赖于合适TGFBRI型受体的存在。参见例如Rosenzweig等(1995)PNAS92:7632-7636。BMPRII的突变与人的肺动脉高压有关。参见OMIM条目600799。

[0079] 缪勒抑制物质受体II(MISRII),AMHR2基因的产物,另称为抗缪勒激素II型受体,是II型TGF-β受体。MISRII结合MIS配体,但需要合适的I型受体(例如ALK3或ALK6)的存在用于信号转导。参见例如Hinck(2012)FEBS Letters586:1860-1870和OMIM条目600956。MISRII参与人的性别分化,并且是人类男性中缪勒激素管消退所必需的。AMH在生殖年龄女性中表达,并且不随生理期或妊娠波动,但发现在卵母细胞数量和质量两者下降时逐步减少,表明AMH可用作卵巢生理学的生物标志物。参见例如Zec等(2011)Biochemia Medica21(3):219-30和OMIM条目600956。

[0080] 在某些方面,本公开内容涉及a)包含TGFβ超家族I型受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7)的胞外域和TGFβ超家族II型受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII)的胞外域的异多聚体复合体,b)包含至少两种TGFβ超家族I型受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7)的胞外域的异多聚体复合体和包含至少两种TGFβ超家族II型受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII)的胞外域的异多聚体复合体,优选可溶性异多聚体复合体拮抗一种或多种TGFβ超

家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、缪勒抑制物质(MIS)和Lefty)引发的胞内信号转导(例如Smad2/3和/或Smad1/5/8信号转导)的用途。如本文所述,所述拮抗剂异多聚体复合体可用于治疗或预防与例如肌肉丢失、肌肉生长不足、神经变性、骨丢失、骨密度降低和/或矿化、骨生长不足、代谢紊乱(例如肥胖症)和红细胞病症(例如贫血)有关的各种病症/病况。

[0081] 具体地讲,本公开内容的数据表明包含TGFβ超家族I型受体多肽的胞外域和TGFβ超家族II型受体多肽的胞外域的异多聚体复合体与其相应的同多聚体复合体相比具有不同的配体结合特异性/特征。

[0082] 用于本说明书的术语一般具有本领域、本公开内容的情况下和使用各术语的具体情况下的普通含义。下面或本说明书其它部分论述了某些术语以在描述本公开内容的组合物和方法和如何制备和使用它们时向从业者提供额外的指导。术语的任何应用的范围或含义从它使用的上下文来看将是显而易见的。

[0083] 术语“异聚体”或“异多聚体”是包含至少一个第一多肽和第二多肽的复合体,其中第二多肽在氨基酸序列中因至少一个氨基酸残基不同于第一多肽。异聚体可包含由第一和第二多肽形成的“异二聚体”或可形成更高阶的结构,其中存在第一和第二多肽以外的多肽。异多聚体的示例性结构包括异二聚体、异三聚体、异四聚体和其它寡聚体结构。异二聚体在本文命名为X:Y或等同于命名为X-Y,其中X表示第一多肽,Y表示第二多肽。更高阶的异聚体和寡聚体结构在本文以相应的方式命名。在某些实施方案中,异多聚体重组的(例如一个或多个多肽组分可以是重组蛋白)、分离的和/或纯化的。

[0084] 在其所有语法形式和拼写变体情况下的“同源的”是指具有“共同进化起源”的两种蛋白质之间的关系,包括来自相同生物物种的超家族的蛋白质以及来自不同的生物物种的同源蛋白质。所述蛋白质(及其编码核酸)具有序列同源性,正如通过其序列相似性所反应的,不论从百分比同一性还是根据特定残基或基序和保守位置的存在来看。然而,在通用用法中和在本申请中,术语“同源”当用副词例如“高度地”修饰时可指序列相似性,并且可与共同进化起源相关或不相关。

[0085] 在其所有语法形式情况下的术语“序列相似性”是指可共有或不共有共同进化起源的核酸或氨基酸序列之间同一性或一致性的程度。

[0086] “百分比(%)序列同一性”就参比多肽(或核苷酸)序列而论定义为在比对序列和引入空位(如有需要)后达到最大百分比序列同一性并且不考虑任何保守取代作为序列同一性的一部分,候选序列中与参比多肽(核苷酸)序列中的氨基酸残基(或核酸)相同的氨基酸残基(或核酸)的百分比。例如可应用可公开获取的计算机软件例如BLAST、BLAST-2、ALIGN或Megalign(DNASTAR)软件,以本领域技术内的各种方法,实现用于确定百分比氨基酸序列同一性的目的的比对。本领域的技术人员可确定用于比对序列的合适参数,包括达到待比较的序列全长内最大比对所需的任何算法。然而,出于本文的目的,%氨基酸(核酸)序列同一性值应用序列比较计算机程序ALIGN-2产生。ALIGN-2序列比较计算机程序由Genentech,Inc.创建,源代码随用户程序说明书保存于U.S.Copyright Office,

Washington D.C.,20559中,其中以登记在美国版权登记号TXU510087下。ALIGN-2程序可公开获自Genentech,Inc.,,South San Francisco,Calif.,或可由源代码编制。ALIGN-2程序可在UNIX操作系统编制以供应用,包括数字UNIX V4.0D。所有序列比较参数通过ALIGN-2程序设置,不再变化。

[0087] 在其所有语法形式情况下,“激动”是指激活蛋白质和/或基因(例如通过激活或扩增该蛋白质的基因表达或通过诱导无活性的蛋白质进入活性状态)或提高蛋白质和/或基因的活性的过程。

[0088] 在其所有语法形式情况下,“拮抗”是指抑制蛋白质和/或基因(例如通过抑制或降低该蛋白质的基因表达或通过诱导活性蛋白质进入无活性状态)或降低蛋白质和/或基因的活性的过程。

[0089] 如本文所用,除非另有说明,否则“基本上不与X结合”意指针对“X”具有大于约10^{-7}、10^{-6}、10^{-5}、10^{-4}或更大(例如通过用于测定K_D的测定法无可检测结合)的K_D的作用剂,其中“X”是特定的作用剂,例如蛋白质或核酸。

[0090] 术语“约”和“大约”在本说明书和权利要求书中与数值连用时表示本领域技术人员熟悉和可接受的准确度区间。一般而言,所述准确度区间为±10%。或者,且特别是在生物系统,术语“约”和“大约”可指给定值的数量级以内的值,优选≤5倍,更优选≤2倍。

[0091] 本文公开的数值范围包括限定该范围的数值。

[0092] 术语“a”和“an”包括复数指示物,除非其中使用该术语的上下文另有明确规定。术语“a”(或“an”)以及术语“一个或多个”和“至少一个”在本文可互换使用。此外,“和/或”当在本文使用时被认为两个或更多个特定特征或组分的每一个与或不与另一个一起的具体公开。因此,术语“和/或”当用于短语例如本文的“A和/或B”时欲包括“A和B”、“A或B”、“A”(单独的)和“B”(单独的)。同样,术语“和/或”在用于短语例如“A、B和/或C”时欲包括以下方面的每一个:A、B和C;A、B、C;A或C;A或B;B或C;A和C;A和B;B和C;A(单独的);B(单独的);和C(单独的)。

[0093] 在整个本说明书中,措词“包含”或变化例如“含有”或“包括”应理解为意味着包括规定的整体或整体群,但不排除任何其它的整体或整体群。

[0094] 2.TGF-β超家族I型受体和II型受体复合体

[0095] 在某些方面,本公开内容涉及包含来自人或其它物种(例如本文所述其它物种)的一种或多种TGF-β超家族I型受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7蛋白,例如SEQ ID NO:14、15、124、126、171、172、413、414、463、464、18、19、136、138、173、174、421、422、465、466、22、23、115、117、175、176、407、408、467、468、26、27、83、84、104、106、177、178、403、404、469、470、30、31、87、88、139、141、179、180、423、424、471、472、34、35、91、92、142、144、181、182、425、426、473、474、38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476)和来自人或其它物种(例如本文所述其它物种)的一种或多种TGF-β超家族II型受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII蛋白,例如SEQ ID NO:9、10、11、118、120、151、152、409、410、451、452、1、2、3、4、5、6、100、102、153、154、401、402、453、454、46、47、71、72、121、123、155、156、411、412、455、456、50、51、75、76、79、80、133、135、161、162、419、420、457、458、42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462)的异多聚体复合体;包含来自人或其它物

种(例如本文所述其它物种)的至少两种不同的TGF-β超家族I型受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7蛋白,例如SEQ ID NO:14、15、124、126、171、172、413、414、463、464、18、19、136、138、173、174、421、422、465、466、22、23、115、117、175、176、407、408、467、468、26、27、83、84、104、106、177、178、403、404、469、470、30、31、87、88、139、141、179、180、423、424、471、472、34、35、91、92、142、144、181、182、425、426、473、474、38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476)的异多聚体复合体;和包含来自人或其它物种(例如本文所述其它物种)的至少两个不同的TGF-β超家族II型受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII蛋白,例如SEQ ID NO:9、10、11、118、120、151、152、409、410、451、452、1、2、3、4、5、6、100、102、153、154、401、402、453、454、46、47、71、72、121、123、155、156、411、412、455、456、50、51、75、76、79、80、133、135、161、162、419、420、457、458、42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462)的异多聚体复合体,其在本文一般称为"异多聚体复合体"或"异多聚体"。优选异多聚体是可溶解的,例如,异多聚体包含至少一种TGFβ超家族I型受体多肽的可溶解部分和至少一种TGFβ超家族II型受体多肽的可溶解部分(结构域)。一般而言,TGFβ超家族I型和II型受体的胞外域相当于I型和II型受体的可溶解部分。因此,在一些实施方案中,本公开内容的异多聚体包含TGFβ超家族I型受体多肽的胞外域(例如一种或多种ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和/或ALK7受体胞外域)和/或TGFβ超家族II型受体多肽的胞外域(例如一种或多种ActRIIA、ActRIIB、TGFBRII、BMPRII和/或MISRII受体胞外域)。本文公开了ALK1、ALK2、ALK3、ALK4、ALK5、ALK6、ALK7、ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII的示例性胞外域,这类序列及其片段、功能变体和修饰形式可按照本公开内容的发明使用(例如异多聚体组合物及其用途)。本公开内容的异多聚体包括例如异二聚体、异三聚体、异四聚体和更高阶寡聚体结构。参见例如图1、2和15-17。在某些优选的实施方案中,本公开内容的异多聚体是异二聚体。

[0096] 限定性结构基序(称为三指毒素折叠)对于配体被I型和II型受体结合是重要的,并且由位于各单体受体胞外域内可变位置内的10、12或14个保守的半胱氨酸残基形成。参见例如Greenwald等(1999)Nat Struct Biol6:18-22;Hinck(2012)FEBS Lett586:1860-1870。通过这些保守半胱氨酸的最外面界定的TGFβ超家族受体的核心配体结合结构域,相当于SEQ ID NO:1(ActRIIB前体)的第29-109位;SEQ ID NO:9(ActRIIA前体)的第30-110位;SEQ ID NO:14(ALK1前体)的第34-95位;SEQ ID NO:18(ALK2前体)的第35-99位;SEQ ID NO:22(ALK3前体)的第61-130位;SEQ ID NO:26和83(ALK4前体)的第34-101位;SEQ ID NO:30和87(ALK5前体)的第36-106位;SEQ ID NO:34(ALK6同种型B前体)的第32-102位;SEQ ID NO:38、305和309(ALK7前体)的第28-92位;SEQ ID NO:42(TGFBRII同种型B前体)的第51-143位;SEQ ID NO:46和71(BMPRII前体)的第34-123位;SEQ ID NO:50、75和79(MISRII前体)的第24-116位;SEQ ID NO:67(TGFBRII同种型A前体)的第44-168位和SEQ ID NO:91(ALK6同种型A前体)的第62-132位。这些半胱氨酸界定的核心序列两侧结构上不太有序的氨基酸可被在任一端截短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或37个残基而又不必改变配体结合。N端和/或C端截短的示例性胞外域包括SEQ ID NO: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和313。

[0097] 在优选的实施方案中，本公开内容的异多聚体结合和/或抑制(拮抗)包括但不限于以下的一种或多种TGF-β超家族配体的活性：BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty。具体地讲，本公开内容的异多聚体复合体可用于拮抗被一种或多种TGFβ超家族配体引发的信号转导(例如Smad2/3和/或Smad1/5/8信号转导)，这可采用例如基于细胞的测定法(例如本文所述的那些)测定。如本文所述，所述拮抗剂异多聚体复合体可用于治疗或预防与例如肌肉丢失、肌肉生长不足、神经变性、骨丢失、骨密度降低和/或矿化、骨生长不足和/或肥胖症有关的病症/病况。在一些实施方案中，本公开内容的异多聚体复合体具有与其相应的同多聚体复合体相比不同的配体结合特异性/特征(例如ALK4:ActRIIB异二聚体与相应的ActRIIB或ALK4同二聚体)。

[0098] 本文所用术语“ActRIIB”是指来自任何物种的激活素受体IIB型(ActRIIB)蛋白家族和通过诱变或其它修饰衍生自这类ActRIIB蛋白的变体。本文对ActRIIB的提及要理解为是提及任一种目前已鉴定的形式。ActRIIB家族的成员一般是跨膜蛋白，由包含富含半胱氨酸区的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0099] 术语“ActRIIB多肽”包括包含ActRIIB家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。在本公开内容以及国际专利申请公布号WO2006/012627、WO2008/097541和Wo2010/151426中提供了这类变体ActRIIB多肽的实例，所述专利申请通过引用以其整体结合到本文中。本文所述所有ActRIIB相关多肽的氨基酸的编号以下文提供的人ActRIIB前体蛋白序列(SEQ ID NO:1)的编号为基础，除非另有特别标注。

[0100] 人ActRIIB前体蛋白序列如下：

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

[0101]

[0102]

[0103] 信号肽用单下划线表示；胞外域用粗体字表示；可能的内源N联糖基化位点用双下划线表示。

[0104] 加工的(成熟的)胞外ActRIIB多肽序列如下：

[0105]

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT(SEQ ID NO:2)。

[0106] 在一些实施方案中,可产生在N端具有"SGR…"序列的蛋白质。胞外域的C端"尾"用单下划线表示。"尾"缺失的序列(一种Δ15序列)如下:GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA(SEQ ID NO:3)。

[0107] 在文献中还报道了在SEQ ID NO:1(A64)的64位具有丙氨酸的ActRIIB形式。参见例如Hilden等(1994)Blood,83(8):2163-2170。申请人断定包含具有A64取代的ActRIIB胞外域的ActRIIB-Fc融合蛋白对激活素和GDF11具有相对低的亲和力。相比之下,在64位具有精氨酸(R64)的同一ActRIIB-Fc融合蛋白对激活素和GDF11具有低纳摩尔至高微微摩尔范围的亲和力。因此,具有R64的序列在本公开内容中用作人ActRIIB的"野生型"参比序列。

[0108] 在64位具有丙氨酸的ActRIIB形式如下:

```
  1  MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
 51  GEQDKRLHCY ASWANSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101  FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151  LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
```

[0109]

```
201  FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251  EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301  LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351  PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401  KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
451  AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
```

[0110]

```
501  TNVDLPPKES SI (SEQ ID NO: 4)
```

[0111] 信号肽用单下划线表示,胞外域用粗体字表示。

[0112] 可变A64形式的加工的(成熟的)胞外ActRIIB多肽序列如下:

[0113]

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT(SEQ ID NO:5)

[0114] 在一些实施方案中,可产生在N端具有"SGR…"序列的蛋白质。胞外域的C端"尾"用单下划线表示。"尾"缺失的序列(Δ15序列)如下:

[0115]

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA(SEQ ID NO:6)

[0116] 编码人ActRIIB前体蛋白的核酸序列见下(SEQ ID NO:7),表示Genbank参比序列NM_001106.3的核苷酸25-1560,其编码ActRIIB前体的氨基酸1-513。所示序列提供64位的精氨酸,并且可经修饰因而提供丙氨酸。信号序列用下划线表示。

[0117]

```
   1  ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGGAT CGCTGTGCGC
  51  CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
 101  CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA
 151  GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
 201  TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
 251  GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
 301  TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC
 351  AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
 401  CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC
 451  CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA
 501  CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC
 551  TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
 601  TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA
 651  GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT
 701  TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC
 751  GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT
 801  CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851  GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901  CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951  TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA
1001  CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051  CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101  TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151  TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201  AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251  GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA
1301  AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351  GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC
1401  TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
1451  CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC
1501  ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC (SEQ ID NO: 7)
```

[0119] 编码加工的胞外人ActRIIB多肽的核酸序列如下(SEQ ID NO:8)。所示序列提供64位的精氨酸,并且可经修饰因而提供丙氨酸。

[0120]

```
   1   GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
  51   GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
 101   AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
 151   ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
 201   TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
 251   GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
 301   GGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
```

(SEQ ID NO: 8)

[0121] 图3说明了人ActRIIB胞外域和人ActRIIA胞外域的氨基酸序列的比对。该比对表示被认为直接接触ActRII配体的两种受体内的氨基酸残基。例如，复合ActRII结构表明ActRIIB-配体结合袋部分由残基Y31、N33、N35、L38经过T41、E47、E50、Q53经过K55、L57、H58、Y60、S62、K74、W78经过N83、Y85、R87、A92和E94经过F101界定。在这些位置上，预期可容许保守突变。

[0122] 另外，ActRIIB在脊椎动物间十分保守，其胞外域的大段序列完全保守。例如，图4表示人ActRIIB胞外域与不同ActRIIB直向同源物相比较的多个序列比对。与ActRIIB结合的许多配体也是高度保守的。因此，从这些比对来看，可预测对正常ActRIIB-配体结合活性是重要的配体结合域内的关键氨基酸位置以及预测可能容许取代而不显著改变正常ActRIIB-配体结合活性的氨基酸位置。因此，可用于本发明公开的方法的有活性的人ActRIIB变体多肽可包括来自另一脊椎动物ActRIIB序列相应位置的一个或多个氨基酸，或可包括与人或其它脊椎动物序列的残基类似的残基。虽无意是限制性的，但以下实例说明该方法界定有活性的ActRIIB变体。人胞外域(SEQ ID NO:2)中的L46在非洲蟾蜍属(非洲蟾蜍属)ActRIIB(SEQ ID NO:505)中为缬氨酸，因此这个位置可变化，任选可变成另一个疏水残基例如V、I或F，或非极性残基例如A。人胞外域中的E52在非洲蟾蜍属中为K，表明这个位点可容许各种各样的改变，包括极性残基，例如E、D、K、R、H、S、T、P、G、Y和可能A。人胞外域中的T93在非洲蟾蜍属中为K，表明在这个位置上容许宽泛的结构变化，其中极性残基是优选的，例如S、K、R、E、D、H、G、P、G和Y。人胞外域中的F108在非洲蟾蜍属中为Y，因此Y或其它疏水基团，例如I、V或L应是容许的。人胞外域中的E111在非洲蟾蜍属中为K，表明在这个位置上可容许带电荷的残基，包括D、R、K和H，以及Q和N。人胞外域中的R112在非洲蟾蜍属中为K，表明在这个位置上容许碱性残基，包括R和H。人胞外域中的119位上是相对保守的，在啮齿动物中似乎为P，而在非洲蟾蜍属中为V，因此在这个位置上基本上应容许任何氨基酸。

[0123] 此外，在本领域中根据结构和功能特征，特别相对于配体结合，对ActRII蛋白进行了表征[Attisano等(1992)Cell68(1):97-108;Greenwald等(1999)Nature Structural Biology6(1):18-22;Allendorph等(2006)PNAS 103(20:7643-7648;Thompson等(2003)The EMBO Journal22(7):1555-1566;以及美国专利号:7,709,605、7,612,041和7,842,663]。除了本文的教导内容以外，这些参考文献提供对如何产生保持一种或多种正常活性(例如配体结合活性)的ActRIIB变体的详尽指导。

[0124] 例如，称为三指毒素折叠的界定性结构基序对于通过I型和II型受体的配体结合是重要的，并且由位于各单体受体胞外域内不同位置上的保守半胱氨酸残基形成[Greenwald等(1999)Nat Struct Biol6:18-22;和Hinck(2012)FEBS Lett586:1860-

1870]。因此，由这些保守半胱氨酸的最外面界定的人ActRIIB的核心配体结合结构域相当于SEQ ID NO:1(ActRIIB前体)的29-109位。因此，这些半胱氨酸界定的核心序列两侧的结构上不太有序的氨基酸在N端可被截短约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或28个残基和/或在C端达约1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25个残基而又不必改变配体结合。N端和/或C端截短的示例性ActRIIB胞外域包括SEQ ID NO:2、3、5和6。

[0125] Attisano等人表明ActRIIB的胞外域C端脯氨酸结的缺失降低受体对激活素的亲和力。含有本文SEQ ID NO:1的氨基酸20-119的ActRIIB-Fc融合蛋白“ActRIIB(20-119)-Fc”，相对于ActRIIB(20-134)-Fc与GDF11和激活素的结合减弱，其包括脯氨酸结区和完整的近膜域(参见例如美国专利号7,842,663)。然而，相对于野生型，ActRIIB(20-129)-Fc蛋白保持类似但略微降低的活性，即使脯氨酸结区遭破坏。

[0126] 因此，预期止于氨基酸134、133、132、131、130和129(相对于SEQ ID NO:1)的ActRIIB胞外域全都有活性，但止于134或133的构建体可能有最大活性。同样，预期残基129-134任一个的突变(相对于SEQ ID NO:1)不会大幅度地改变配体结合亲和力。支持这一点的是，本领域已知P129和P130的突变(相对于SEQ ID NO:1)基本上不减弱配体结合。因此，本公开内容的ActRIIB多肽可早在氨基酸109(最后的半胱氨酸)处终止，然而，预期止于109和119或其之间(例如109、110、111、112、113、114、115、116、117、118或119)的形式的配体结合减弱。氨基酸119(相对于本文的SEQ ID NO:1)是不太保守的，因此容易改变或截短。止于128(相对于SEQ ID NO:1)或稍后的ActRIIB多肽可保持配体结合活性。相对于SEQ ID NO:1，止于119和127或其之间(例如119、120、121、122、123、124、125、126或127)的ActRIIB多肽可具有中间结合能力。这些形式的任一个对于使用是合乎需要的，这取决于临床或实验背景。

[0127] 在ActRIIB的N端，预期始于氨基酸29或之前(相对于SEQ ID NO:1)的蛋白质可保留配体结合活性。氨基酸29表示起始半胱氨酸。24位(相对于SEQ ID NO:1)的丙氨酸至天冬酰胺突变引入N-联糖基化序列而不实质性地影响配体结合[美国专利号7,842,663]。这证实了信号切割肽和半胱氨酸交联区(相当于氨基酸20-29)之间区域中的突变是绝对容许的。具体地讲，始于20、21、22、23和24位(相对于SEQ ID NO:1)的ActRIIB多肽可保持大致的配体结合活性，而且还预期始于25、26、27、28和29位(相对于SEQ ID NO:1)的ActRIIB多肽保留配体结合活性。例如美国专利号7,842,663表明，出人意料地，始于22、23、24或25的ActRIIB构建体可具有最大活性。

[0128] 总之，ActRIIB的活性部分(例如配体结合部分)的通式包含SEQ ID NO:1的氨基酸29-109。因此ActRIIB多肽例如可包含与始于相当于SEQ ID NO:1的氨基酸20-29任一个的残基(例如始于氨基酸20、21、22、23、24、25、26、27、28或29的任一个)和止于相当于SEQ ID NO:1的氨基酸109-134任一个的位置(例如止于氨基酸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或134的任一个)的ActRIIB的部分有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列或基本上由其组成或由其组成。其它实例包括始于SEQ ID NO:1的20-29位(例如20、21、22、23、24、25、26、27、28或29位的任一个)或21-29位(例如21、22、23、24、25、26、27、28或29位的任一

个)和止于SEQ ID NO:1的119-134位(例如119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134位的任一个)、119-133位(例如119、120、121、122、123、124、125、126、127、128、129、130、131、132或133位的任一个)、129-134位(例如129、130、131、132、133或134位的任一个)或129-133位(例如129、130、131、132或133位的任一个)的多肽。其它实例包括始于SEQ ID NO:1的20-24位(例如位的任一个20、21、22、23或24)、21-24位(例如位的任一个21、22、23或24)或22-25位(例如22、22、23或25位的任一个)和止于SEQ ID NO:1的109-134位(例如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或134位的任一个)、119-134位(例如119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134位的任一个)或129-134位(例如129、130、131、132、133或134位的任一个)的构建体。还预期这些范围内的变体,特别是与SEQ ID NO:1的相应部分有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的那些。

[0129] 本文所述变异可以不同方式组合。在一些实施方案中,ActRIIB变体包含在配体结合袋中不超过1、2、5、6、7、8、9、10或15个保守氨基酸变化和在配体结合袋的40、53、55、74、79和/或82位上0、1或多个非保守变化。结合袋以外的部位(在其中可特别好地容许变化性)包括胞外域的氨基和羧基端(如上所述)和42-46和65-73位(相对于SEQ ID NO:1)。在A64背景下,65位(N65A)处天冬酰胺向丙氨酸变化实际上提高配体结合,因此预期在R64背景下对配体结合无有害作用[美国专利号7,842,663]。这个变化可能排除A64背景下N65的糖基化,因此表明,这个区的重大变化可能是容许的。虽然R64A变化不太容许,但R64K是极为容许的,因此可容许64位处另一个碱性残基,例如H[美国专利号7,842,663]。另外,本领域描述的诱变程序的结果表明,在ActRIIB中常常有有利于保守的氨基酸位置。相对于SEQ ID NO:1,这些包括80位(酸性或疏水氨基酸)、78位(疏水,特别是色氨酸)、37位(酸性,特别是天冬氨酸或谷氨酸)、56位(碱性氨基酸)、60位(疏水氨基酸,特别是苯丙氨酸或酪氨酸)。因此,本公开内容提供在ActRIIB多肽中可为保守的氨基酸构架。可能需要保守的其它位置如下:52位(酸性氨基酸)、55位(碱性氨基酸)、81位(酸性)、98位(极性或带电荷的,特别是E、D、R或K),均相对于SEQ ID NO:1。

[0130] 在某些实施方案中,本公开内容涉及包含至少一种ActRIIB多肽的异多聚体,其包括其片段、功能变体和修饰形式。优选用于本公开内容的ActRIIB多肽是可溶解的(例如ActRIIB的胞外域)。在其它优选的实施方案中,用于本公开内容的ActRIIB多肽与一种或多种TGF-β超家族配体结合。因此,在一些实施方案中,用于本公开内容的ActRIIB多肽抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如抑制Smad信号转导)。在一些实施方案中,本公开内容的异多聚体包含至少一种ActRIIB多肽,其包含始于相当于SEQ ID NO:1的氨基酸20-29的残基(例如始于氨基酸20、21、22、23、24、25、26、27、28或29的任一个)和止于相当于SEQ ID NO:1的氨基酸109-134的位置(例如止于氨基酸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或134的任一个)的ActRIIB的部分有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列、基本由其组成或由其组成。在某些优选的实施方案中,本公开内容的异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1的氨基酸29-109有至少70%、75%、80%、85%、86%、

87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列、由其组成或基本由其组成。在其它优选的实施方案中，本公开内容的异多聚体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1的氨基酸25-131有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的的氨基酸序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的异多聚体包含至少一种ActRIIB多肽，其与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、97％、98％、99％或100％同一性。在某些实施方案中，本公开内容的异多聚体包含至少一种ActRIIB多肽，其中相当于SEQ ID NO:1的L79的氨基酸位置不是酸性氨基酸(即不是天然存在的D或E氨基酸残基或人工酸性氨基酸)。

[0131] 在某些实施方案中，本公开内容涉及包含ActRIIA多肽的蛋白质复合体。本文所用术语“ActRIIA”是指来自任何物种的激活素受体IIA型(ActRIIA)蛋白的家族和通过诱变或其它修饰衍生于这类ActRIIA蛋白的变体。本文对ActRIIA的提及要理解为是提及目前已鉴定形式的任一种。ActRIIA家族的成员一般是跨膜蛋白，由包含富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0132] 术语“ActRIIA多肽”包括包含ActRIIA家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。在整个本公开内容以及国际专利申请公布号WO2006/012627(其通过引用以其整体结合到本文中)中提供这类变体ActRIIA多肽的实例。本文所述的所有ActRIIA相关多肽的氨基酸的编号以下文提供的人ActRIIA前体蛋白序列(SEQ ID NO:9)的编号为基础，除非另有特别标注。

[0133] 人ActRIIA前体蛋白序列如下：

[0134]

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

[0135]

[0136] 信号肽用单下划线表示；胞外域用粗体字表示；可能的内源N联糖基化位点用双下划线表示。

[0137] 加工的胞外人ActRIIA多肽序列如下：

[0138]

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPP(SEQ ID NO:10)

[0139] 胞外域的C端“尾”用单下划线表示。“尾”缺失的序列(Δ15序列)如下：

[0140]

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDS PEVYFCCCEGNMCNEKFSYFPEM(SEQ ID NO:11)

[0141] 编码人ActRIIA前体蛋白的核酸序列见下(SEQ ID NO:12)，相当于Genbank参比序列NM_001616.4的核苷酸159-1700。信号序列用下划线表示。

```
   1  ATGGGAGCTG CTGCAAAGTT GGCGTTTGCC GTCTTTCTTA TCTCCTGTTC
  51  TTCAGGTGCT ATACTTGGTA GATCAGAAAC TCAGGAGTGT CTTTTCTTTA
 101  ATGCTAATTG GGAAAAAGAC AGAACCAATC AAACTGGTGT TGAACCGTGT
 151  TATGGTGACA AAGATAAACG GCGGCATTGT TTTGCTACCT GGAAGAATAT
 201  TTCTGGTTCC ATTGAAATAG TGAAACAAGG TTGTTGGCTG GATGATATCA
 251  ACTGCTATGA CAGGACTGAT TGTGTAGAAA AAAAAGACAG CCCTGAAGTA
 301  TATTTTTGTT GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATTT
 351  TCCGGAGATG GAAGTCACAC AGCCCACTTC AAATCCAGTT ACACCTAAGC
 401  CACCCTATTA CAACATCCTG CTCTATTCCT TGGTGCCACT TATGTTAATT
[0142]
 451  GCGGGGATTG TCATTTGTGC ATTTTGGGTG TACAGGCATC ACAAGATGGC
 501  CTACCCTCCT GTACTTGTTC CAACTCAAGA CCCAGGACCA CCCCCACCTT
 551  CTCCATTACT AGGTTTGAAA CCACTGCAGT TATTAGAAGT GAAAGCAAGG
 601  GGAAGATTTG GTTGTGTCTG GAAAGCCCAG TTGCTTAACG AATATGTGGC
 651  TGTCAAAATA TTTCCAATAC AGGACAAACA GTCATGGCAA AATGAATACG
 701  AAGTCTACAG TTTGCCTGGA ATGAAGCATG AGAACATATT ACAGTTCATT
 751  GGTGCAGAAA AACGAGGCAC CAGTGTTGAT GTGGATCTTT GGCTGATCAC
 801  AGCATTTCAT GAAAAGGGTT CACTATCAGA CTTTCTTAAG GCTAATGTGG
 851  TCTCTTGGAA TGAACTGTGT CATATTGCAG AAACCATGGC TAGAGGATTG
 901  GCATATTTAC ATGAGGATAT ACCTGGCCTA AAAGATGGCC ACAAACCTGC
 951  CATATCTCAC AGGGACATCA AAAGTAAAAA TGTGCTGTTG AAAAACAACC
1001  TGACAGCTTG CATTGCTGAC TTTGGGTTGG CCTTAAAATT TGAGGCTGGC
1051  AAGTCTGCAG GCGATACCCA TGGACAGGTT GGTACCCGGA GGTACATGGC
1101  TCCAGAGGTA TTAGAGGGTG CTATAAACTT CCAAAGGGAT GCATTTTTGA
1151  GGATAGATAT GTATGCCATG GGATTAGTCC TATGGGAACT GGCTTCTCGC
1201  TGTACTGCTG CAGATGGACC TGTAGATGAA TACATGTTGC CATTTGAGGA
[0143]
1251  GGAAATTGGC CAGCATCCAT CTCTTGAAGA CATGCAGGAA GTTGTTGTGC
1301  ATAAAAAAAA GAGGCCTGTT TTAAGAGATT ATTGGCAGAA ACATGCTGGA
1351  ATGGCAATGC TCTGTGAAAC CATTGAAGAA TGTTGGGATC ACGACGCAGA
1401  AGCCAGGTTA TCAGCTGGAT GTGTAGGTGA AAGAATTACC CAGATGCAGA
1451  GACTAACAAA TATTATTACC ACAGAGGACA TTGTAACAGT GGTCACAATG
1501  GTGACAAATG TTGACTTTCC TCCCAAAGAA TCTAGTCTA
```

(SEQ ID NO: 12)

[0144] 编码加工的胞外ActRIIA多肽的核酸序列如下：

[0145]

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

[0146] 有活性的(例如配体结合)ActRIIA多肽的通式是包含始于SEQ ID NO:9的氨基酸30和止于氨基酸110的多肽的通式。因此,本公开内容的ActRIIA多肽可包含与SEQ ID NO:9的氨基酸30-110有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性的多肽。任选本公开内容的ActRIIA多肽包含任选分别始于范围为1-5(例如1、2、3、4或5)或3-5(例如3、4或5)的位置和止于范围为110-116(例如110、111、112、113、114、115或116)或110-115(例如110、111、112、113、114或115)的位置的SEQ ID NO:9的氨基酸12-82有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性和相对于SEQ ID NO:9包含在配体结合袋中不超过1、2、5、10或15个保守氨基酸变化和在配体结合袋的40、53、55、74、79和/或82位的0、1或多个非保守变化的多肽。

[0147] 在某些实施方案中,本公开内容涉及包含至少一种ActRIIA多肽、其包括其片段、功能变体和修饰形式的异多聚体复合体。优选用于本公开内容发明(例如包含ActRIIA多肽的异多聚体复合体及其用途)的ActRIIA多肽是可溶解的(例如ActRIIA的胞外域)。在其它优选的实施方案中,用于本发明本公开内容的ActRIIA多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,本公开内容的异多聚体复合体包含与SEQ ID NO:9、10、11、118、120、409或410任一个的氨基酸序列有至少70%、75%、80%、85%、90%、95%、97%、98%或99%同一性的至少一种ActRIIA多肽。在一些实施方案中,本公开内容的异多聚体复合体包含与SEQ ID NO:9、10、11、118、120、409或410任一个的氨基酸序列有至少70%、75%、80%、85%、90%、95%、97%、98%或99%同一性的至少一种ActRIIA多肽、由其组成或基本由其组成。

[0148] 在某些方面,本公开内容涉及包含TGFBRII多肽的蛋白质复合体。本文所用术语“TGFBRII”是指来自任何物种的转化生长因子-β受体II(TGFBRII)蛋白的家族和通过诱变或其它修饰衍生自所述蛋白质的变体。本文对TGFBRII的提及要理解为是提及目前已鉴定形式的任一种。TGFBRII家族的成员一般是跨膜蛋白,由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0149] 术语“TGFBRII多肽”包括包含TGFBRII家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。所有本文所述的TGFBRII相关多肽的氨基酸的编号以下面人TGFBRII前体蛋白序列(SEQ ID NO:42)的编号为基础,除非另有特别标注。

[0150] 典型的人TGFBRII前体蛋白序列(NCBIRefSeqNP_003233.4)如下:

[0151]

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

[0152] 信号肽用单下划线表示,胞外域用粗体字表示。

[0153] 加工的胞外TGFBRII多肽序列如下:

[0154]

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQ(SEQ ID NO:43)

[0155] 编码TGFBRII前体蛋白的核酸序列见下(SEQ ID NO:44),相当于Genbank参比序列NM_003242.5的核苷酸383-2083。信号序列用下划线表示。

[0156] ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGC
ACGATCCCACCGCACGTTCAGA
AGTCGGTTAATAACGACATGATAGTCACTGACAACAACGGTG
CAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATT
TTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAG
CATCACCTCCATCTGTGAGAAGCCACAGGAAGTCTGTGTGGC
TGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGT
TTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAA
GATGCTGCTTCTCCAAAGTGCATTATGAAGGAAAAAAAAAAG
CCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGT
GCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCA
ATCCTGACTTGTTGCTAGTCATATTTCAA
GTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGTCATCATCATCTTCTACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCAACCTGGGAAACCGGCAAGACGCGGAAGCTCATGGAGTTCAGCGAGCACTGTGCCATCATCCTGGAAGATGACCGCTCTGACATCAGCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCCATTGAGCTGGACACCCTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGCAGAACACTTCAGAGCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCCTCTTGGAAGACAGAGAAGGACATCTTCT

CAGACATCAATCTGAAGCATGAGAACATACTCCAGTTCCTGACGGCTGAGGAGCGGAAGACGGAGTTGGGGAAACAA
TACTGGCTGATCACCGCCTTCCACGCCAAGGGCAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGA
CCTGCGCAAGCTGGGCAGCTCCCTCGCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATGTGGGAGGCCCA
AGATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGAACGACCTAACCTGCTGCCTGTGTGAC
TTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCTGTGGATGACCTGGCTAACAGTGGGCAGGTGGGAACTGCAAG
ATACATGGCTCCAGAAGTCCTAGAATCCAGGATGAATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACT
CCATGGCTCTGGTGCTCTGGGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAGCCTCCATTT
GGTTCCAAGGTGCGGGAGCACCCCTGTGTCGAAAGCATGAAGGACAACGTGTTGAGAGATCGAGGGCGACCAGAAAT
TCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGGTGTGTGAGACGTTGACTGAGTGCTGGGACCACGACCCAG
AGGCCCGTCTCACAGCCCAGTGTGTGGCAGAACGCTTCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGC
TGCTCGGAGGAGAAGATTCCTGAAGACGGCTCCCTAAACACTACCAAA(SEQ ID NO:44)

[0157] 编码加工的胞外TGFBRII多肽的核酸序列如下：

[0158]

ACGATCCCACCGCACGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACAACGGTGCAGTCAAGTTTCC
ACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCA
CCTCCATCTGTGAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACA
GTTTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGA
AAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCAG
AAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAA(SEQ ID NO:45)

[0159] TGFBRII的可变同种型,同种型A(NP_001020018.1)如下：

1 MGRGLLRGLW PLHIVLWTRI AS**TIPPHVQK SDVEMEAQKD EIICPSCNRT**

51 **AHPLRHINND MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS**

101 **CEKPQEVCV AVWRKNDENI TLETVCHDPK LPYHDFILED AASPKCIMKE**

151 **KKKPGETFFM CSCSSDECND NIIFSEEYNT SNPDLLLVIF Q**VTGISLLPP

201 LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEHCAIILEDDRS

251 DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA

[0160] 301 VKIFPYEEYA SWKTEKDIFS DINLKHENIL QFLTAEERKT ELGKQYWLIT

351 AFHAKGNLQE YLTRHVISWE DLRKLGSSLA RGIAHLHSDH TPCGRPKMPI

401 VHRDLKSSNI LVKNDLTCCL CDFGLSLRLD PTLSVDDLAN SGQVGTARYM

451 APEVLESRMN LENVESFKQT DVYSMALVLW EMTSRCNAVG EVKDYEPPFG

501 SKVREHPCVE SMKDNVLRDR GRPEIPSFWL NHQGIQMVCE TLTECWDHDP

551 EARLTAQCVA ERFSELEHLD RLSGRSC 参见 KIPEDGSLNT TK

SEQ ID NO: 67)

[0161] 信号肽用单下划线表示,胞外域用粗体字表示。

[0162] 加工的胞外TGFBRII多肽序列(同种型A)如下：

[0163]

TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSE
EYNTSNPDLLLVIFQ(SEQ ID NO:68)

[0164] 编码TGFBRII前体蛋白(同种型A)的核酸序列见下(SEQ ID NO:69)，相当于Genbank参比序列NM_001024847.2的核苷酸383-2158。信号序列用下划线表示。

[0165] ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGC
ACGATCCCACCGCACGTTCAGA
AGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAATCATCT
GCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTA
ATAACGACATGATAGTCACTGACAACAACGGTGCAGTCAAGT
TTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTG
TGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTC
CATCTGTGAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAG
AAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGA
CCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCT
TCTCCAAAGTGCATTATGAAGGAAAAAAAAAAGCCTGGTGAG
ACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGACA
ACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTT
GTTGCTAGTCATATTTCAA
GTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGTCATCATCATCTTCTACTGCTACCGCGTTAA
CCGGCAGCAGAAGCTGAGTTCAACCTGGGAAACCGGCAAGACGCGGAAGCTCATGGAGTTCAGCGAGCACTGTGCCA
TCATCCTGGAAGATGACCGCTCTGACATCAGCTCCACGTGTGCCAACAACATCAACCACAACACAGAGCTGCTGCCC
ATTGAGCTGGACACCCTGGTGGGGAAAGGTCGCTTTGCTGAGGTCTATAAGGCCAAGCTGAAGCAGAACACTTCAGA
GCAGTTTGAGACAGTGGCAGTCAAGATCTTTCCCTATGAGGAGTATGCCTCTTGGAAGACAGAGAAGGACATCTTCT
CAGACATCAATCTGAAGCATGAGAACATACTCCAGTTCCTGACGGCTGAGGAGCGGAAGACGGAGTTGGGGAAACAA
TACTGGCTGATCACCGCCTTCCACGCCAAGGGCAACCTACAGGAGTACCTGACGCGGCATGTCATCAGCTGGGAGGA
CCTGCGCAAGCTGGGCAGCTCCCTCGCCCGGGGGATTGCTCACCTCCACAGTGATCACACTCCATGTGGGAGGCCCA
AGATGCCCATCGTGCACAGGGACCTCAAGAGCTCCAATATCCTCGTGAAGAACGACCTAACCTGCTGCCTGTGTGAC
TTTGGGCTTTCCCTGCGTCTGGACCCTACTCTGTCTGTGGATGACCTGGCTAACAGTGGGCAGGTGGGAACTGCAAG
ATACATGGCTCCAGAAGTCCTAGAATCCAGGATGAATTTGGAGAATGTTGAGTCCTTCAAGCAGACCGATGTCTACT
CCATGGCTCTGGTGCTCTGGGAAATGACATCTCGCTGTAATGCAGTGGGAGAAGTAAAAGATTATGAGCCTCCATTT
GGTTCCAAGGTGCGGGAGCACCCCTGTGTCGAAAGCATGAAGGACAACGTGTTGAGAGATCGAGGGCGACCAGAAAT
TCCCAGCTTCTGGCTCAACCACCAGGGCATCCAGATGGTGTGTGAGACGTTGACTGAGTGCTGGGACCACGACCCAG
AGGCCCGTCTCACAGCCCAGTGTGTGGCAGAACGCTTCAGTGAGCTGGAGCATCTGGACAGGCTCTCGGGGAGGAGC
TGCTCGGAGGAGAAGATTCCTGAAGACGGCTCCCTAAACACTACCAAA(SEQ ID NO:69)

[0166] 编码加工的胞外TGFBRII多肽(同种型A)的核酸序列如下：

[0167]
ACGATCCCACCGCACGTTCAGAAGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAATCATCTGCCCCAGCTGTAA
TAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATAGTCACTGACAACAACGGTGCAGTCAAGTTTCCAC
AACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACC

TCCATCTGTGAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGT TTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAA AAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCAGAA GAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAA(SEQ ID NO:70)。

[0168] 前述TGFβRII同种型的任一种(SEQ ID NO:42、43、67和68)可在位于接近TGFβRIIECD的C端的谷氨酸残基对(SEQ ID NO:42的151和152位;SEQ ID NO:43的129和130位;SEQ ID NO:67的176和177位;或SEQ ID NO:68的154和155位)之间掺入36个氨基酸的插入(SEQ ID NO:95),正如TGFβRII同种型C中天然存在的一样(Konrad等,BMCGenomics8:318,2007)。

[0169] GRCKIRHIGS NNRLQRSTCQ NTGWESAHVM KTPGFR(SEQ ID NO:95)

[0170] 在某些实施方案中,本公开内容涉及包含至少一种TGFBRII多肽的异多聚体复合体,其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的TGFBRII多肽(例如包含TGFBRII多肽的异多聚体复合体及其用途)是可溶解的(例如TGFBRII的胞外域)。在其它优选的实施方案中,用于本发明本公开内容的TGFBRII多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,本公开内容的异多聚体复合体包含含或不含上述SEQ ID NO:95的插入、与SEQ ID NO:42、43、67或68的氨基酸序列有至少70%、75%、80%、85%、90%、95%、97%、98%或99%同一性的至少一种TGFBRII多肽。在一些实施方案中,本公开内容的异多聚体复合体由含或不含SEQ ID NO:95的插入、与SEQ ID NO:42、43、67或68的氨基酸序列有至少70%、75%、80%、85%、90%、95%、97%、98%或99%同一性的至少一种TGFBRII多肽组成或基本由其组成。

[0171] 在某些方面,本公开内容涉及包含BMPRII多肽的蛋白质复合体。本文所用术语"BMPRII"是指来自任何物种的骨形态发生蛋白受体II型(BMPRII)蛋白的家族和通过诱变或其它修饰衍生于所述BMPRII蛋白的变体。本文对BMPRII的提及要理解为是提及目前已鉴定形式的任一种。BMPRII家族的成员一般是跨膜蛋白,由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0172] 术语"BMPRII多肽"包括包含BMPRII家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。所有本文所述BMPRII相关多肽的氨基酸的编号以下面人BMPRII前体蛋白序列(SEQ ID NO:46)的编号为基础,除非另有特别标注。

[0173] 典型的人BMPRII前体蛋白序列(NCBI Ref Seq NP_001195.2)如下:

```
  1 MTSSLQRPWR VPWLPWTILL VSTAAASQNQ ERLCAFKDPY QQDLGIGESR
 51 ISHENGTILCSKGSTCYGLW EKSKGDINLV KQGCWSHIGD PQECHYEECV
101 VTTTPPSIQN GTYRFCCCSTDLCNVNFTEN FPPPDTTPLS PPHSFNRDET
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[0174]
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151 IIIALASVSV LAVLIVALCF GYRMLTGDRKQGLHSMNMME AAASEPSLDL
201 DNLKLLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNFINEKNIYRVP
251 LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS
301 SCRLAHSVTR GLAYLHTELP RGDHYKPAIS HRDLNSRNVL VKNDGTCVIS
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351 DFGLSMRLTGNRLVRPGEED NAAISEVGTI RYMAPEVLEG AVNLRDCESA

401 LKQVDMYALG LIYWEIFMRCTDLFPGESVP EYQMAFQTEV GNHPTFEDMQ

451 VLVSREKQRP KFPEAWKENS LAVRSLKETIEDCWDQDAEA RLTAQCAEER

501 MAELMMIWER NKSVSPTVNP MSTAMQNERN LSHNRRVPKIGPYPDYSSSS

551 YIEDSIHHTD SIVKNISSEH SMSSTPLTIG EKNRNSINYE RQQAQARIPS

601 PETSVTSLST NTTTTNTTGL TPSTGMTTIS EMPYPDETNL HTTNVAQSIG

651 PTPVCLQLTEEDLETNKLDP KEVDKNLKES SDENLMEHSL KQFSGPDPLS

[0175] 701 STSSSLLYPL IKLAVEATGQQDFTQTANGQ ACLIPDVLPT QIYPLPKQQN

751 LPKRPTSLPL NTKNSTKEPR LKFGSKHKSNLKQVETGVAK MNTINAAEPH

801 VVTVTMNGVA GRNHSVNSHA ATTQYANGTV LSGQTTNIVTHRAQEMLQNQ

851 FIGEDTRLNI NSSPDEHEPL LRREQQAGHD EGVLDRLVDR RERPLEGGRT

901 NSNNNNSNPC SEQDVLAQGV PSTAADPGPS KPRRAQRPNS LDLSATNVLD

951 GSSIQIGESTQDGKSGSGEK IKKRVKTPYS LKRWRPSTWV ISTESLDCEV

1001 NNNGSNRAVH SKSSTAVYLA EGGTATTMVS KDIGMNCL

(SEQ ID NO: 46)

[0176] 信号肽用单下划线表示,胞外域用粗体字表示。

[0177] 加工的胞外BMPRII多肽序列如下:

[0178]

SQNQERLCAFKDPYQQDLGIGESRISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTT TPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET(SEQ ID NO:47)

[0179] 编码BMPRII前体蛋白的核酸序列见下(SEQ ID NO:48),相当于Genbank参比序列NM_001204.6的核苷酸1149-4262。信号序列用下划线表示。

[0180]

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGTCAGCACTGCGGCTGC TTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGGTGAGAGTAGAATCT CTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATA AATCTTGTAAAACAAGGATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTAC CACTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTG AGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACAATAATCATTGCT TTGGCATCAGTCTCTGTATTAGCTGTTTTGATAGTTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAA ACAAGGTCTTCACAGTATGAACATGATGGAGGCAGCAGCATCCGAACCCTCTCTTGATCTAGATAATCTGAAACTGT TGGAGCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTGCTGTAAAAGTG TTTTCCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGAGTGCCTTTGATGGAACATGACAACAT TGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGCAGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATC CCAATGGATCTTTATGCAAGTATTTAAGTCTCCACACAAGTGACTGGGTAAGCTCTTGCCGTCTTGCTCATTCTGTT ACTAGAGGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCATCGAGATTT AAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACTTTGGACTGTCCATGAGGCTGACTG GAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCAGCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCA GAAGTGCTAGAAGGAGCTGTGAACTTGAGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGACT

AATCTATTGGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATGGCTTTTC
AGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAGGTTCTCGTGTCTAGGGAAAAACAGAGACCCAAGTTC
CCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCACTCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGC
AGAGGCTCGGCTTACTGCACAGTGTGCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTG
TGAGCCCAACAGTCAATCCAATGTCTACTGCTATGCAGAATGAACGCAACCTGTCACATAATAGGCGTGTGCCAAAA
ATTGGTCCTTATCCAGATTATTCTTCCTCCTCATACATTGAAGACTCTATCCATCATACTGACAGCATCGTGAAGAA
TATTTCCTCTGAGCATTCTATGTCCAGCACACCTTTGACTATAGGGGAAAAAAACCGAAATTCAATTAACTATGAAC
GACAGCAAGCACAAGCTCGAATCCCCAGCCCTGAAACAAGTGTCACCAGCCTCTCCACCAACACAACAACCACAAAC
ACCACAGGACTCACGCCAAGTACTGGCATGACTACTATATCTGAGATGCCATACCCAGATGAAACAAATCTGCATAC
CACAAATGTTGCACAGTCAATTGGGCCAACCCCTGTCTGCTTACAGCTGACAGAAGAAGACTTGGAAACCAACAAGC
TAGACCCAAAAGAAGTTGATAAGAACCTCAAGGAAAGCTCTGATGAGAATCTCATGGAGCACTCTCTTAAACAGTTC
AGTGGCCCAGACCCACTGAGCAGTACTAGTTCTAGCTTGCTTTACCCACTCATAAAACTTGCAGTAGAAGCAACTGG
ACAGCAGGACTTCACACAGACTGCAAATGGCCAAGCATGTTTGATTCCTGATGTTCTGCCTACTCAGATCTATCCTC
TCCCCAAGCAGCAGAACCTTCCCAAGAGACCTACTAGTTTGCCTTTGAACACCAAAAATTCAACAAAAGAGCCCCGG
CTAAAATTTGGCAGCAAGCACAAATCAAACTTGAAACAAGTCGAAACTGGAGTTGCCAAGATGAATACAATCAATGC
AGCAGAACCTCATGTGGTGACAGTCACCATGAATGGTGTGGCAGGTAGAAACCACAGTGTTAACTCCCATGCTGCCA
CAACCCAATATGCCAATGGGACAGTACTATCTGGCCAAACAACCAACATAGTGACACATAGGGCCCAAGAAATGTTG
CAGAATCAGTTTATTGGTGAGGACACCCGGCTGAATATTAATTCCAGTCCTGATGAGCATGAGCCTTTACTGAGACG
AGAGCAACAAGCTGGCCATGATGAAGGTGTTCTGGATCGTCTTGTGGACAGGAGGGAACGGCCACTAGAAGGTGGCC
GAACTAATTCCAATAACAACAACAGCAATCCATGTTCAGAACAAGATGTTCTTGCACAGGGTGTTCCAAGCACAGCA
GCAGATCCTGGGCCATCAAAGCCCAGAAGAGCACAGAGGCCTAATTCTCTGGATCTTTCAGCCACAAATGTCCTGGA
TGGCAGCAGTATACAGATAGGTGAGTCAACACAAGATGGCAAATCAGGATCAGGTGAAAAGATCAAGAAACGTGTGA
AAACTCCCTATTCTCTTAAGCGGTGGCGCCCCTCCACCTGGGTCATCTCCACTGAATCGCTGGACTGTGAAGTCAAC
AATAATGGCAGTAACAGGGCAGTTCATTCCAAATCCAGCACTGCTGTTTACCTTGCAGAAGGAGGCACTGCTACAAC
CATGGTGTCTAAAGATATAGGAATGAACTGTCTG(SEQ ID NO:48)

[0181] 编码胞外BMPRII多肽的核酸序列如下：

[0182]

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGGTGAGAGTAGAATCTC
TCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAA
ATCTTGTAAAACAAGGATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACC
ACTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGA
GAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA(SEQ ID NO:
49)

[0183] BMPRII的可变同种型,同种型2(GenBank:AAA86519.1)如下：

1 MTSSLQRPWR VPWLPWTILL VSTAAA**SQNQ ERLCAFKDPY QQDLGIGESR**

51 ISHENGTILC SKGSTCYGLW EKSKGDINLV KQGCWSHIGD PQECHYEECV

101 VTTTPPSIQN GTYRFCCCST DLCNVNFTEN FPPPDTTPLS PPHSFNRDET

151 IIIALASVSV LAVLIVALCF GYRMLTGDRK QGLHSMNMME AAASEPSLDL

201 DNLKLLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNF INEKNIYRVP

[0184] 251 LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS

301 SCRLAHSVTR GLAYLHTELP RGDHYKPAIS HRDLNSRNVL VKNDGTCVIS

351 DFGLSMRLTG NRLVRPGEED NAAISEVGTI RYMAPEVLEG AVNLRDCESA

401 LKQVDMYALG LIYWEIFMRC TDLFPGESVP EYQMAFQTEV GNHPTFEDMQ

451 VLVSREKQRP KFPEAWKENS LAVRSLKETI EDCWDQDAEA RLTAQCAEER

501 MAELMMIWER NKSVSPTVNP MSTAMQNERR (SEQ ID NO: 71)

[0185] 信号肽用单下划线表示,胞外域用粗体字表示。

[0186] 加工的胞外BMPRII多肽序列(同种型2)如下:

[0187]

SQNQERLCAFKDPYQQDLGIGESRISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTT TPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET(SEQ ID NO:72)

[0188] 编码人BMPRII前体蛋白(同种型2)的核酸序列见下(SEQ ID NO:73),相当于Genbank参比序列U25110.1的核苷酸163-1752。信号序列用下划线表示。

[0189]

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGTCAGCACTGCGGCTGC TTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGGTGAGAGTAGAATCT CTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATA AATCTTGTAAAACAAGGATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTAC CACTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTG AGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACAATAATCATTGCT TTGGCATCAGTCTCTGTATTAGCTGTTTTGATAGTTGCCTTATGCTTTGGATACAGAATGTTGACAGGAGACCGTAA ACAAGGTCTTCACAGTATGAACATGATGGAGGCAGCAGCATCCGAACCCTCTCTTGATCTAGATAATCTGAAACTGT TGGAGCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGTCCAGTTGCTGTAAAAGTG TTTTCCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGAGTGCCTTTGATGGAACATGACAACAT TGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGCAGATGGACGCATGGAATATTTGCTTGTGATGGAGTACTATC CCAATGGATCTTTATGCAAGTATTTAAGTCTCCACACAAGTGACTGGGTAAGCTCTTGCCGTCTTGCTCATTCTGTT ACTAGAGGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTATAAACCTGCAATTTCCCATCGAGATTT AAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGTGACTTTGGACTGTCCATGAGGCTGACTG GAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCAGCCATAAGCGAGGTTGGCACTATCAGATATATGGCACCA GAAGTGCTAGAAGGAGCTGTGAACTTGAGGGACTGTGAATCAGCTTTGAAACAAGTAGACATGTATGCTCTTGGACT AATCTATTGGGAGATATTTATGAGATGTACAGACCTCTTCCCAGGGGAATCCGTACCAGAGTACCAGATGGCTTTTC AGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAGGTTCTCGTGTCTAGGGAAAAACAGAGACCCAAGTTC CCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCACTCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGC AGAGGCTCGGCTTACTGCACAGTGTGCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTG

TGAGCCCAACAGTCAATCCAATGTCTACTGCTATGCAGAATGAACGTAGG(SEQ ID NO:73)

[0190] 编码胞外BMPRII多肽(同种型2)的核酸序列如下：

[0191]

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGGTGAGAGTAGAATCTC
TCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGACATAA
ATCTTGTAAAACAAGGATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACC
ACTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGA
GAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA(SEQ ID NO:
74)

[0192] 在某些实施方案中，本公开内容涉及包含至少一种BMPRII多肽的异多聚体复合体，其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的BMPRII多肽(例如包含BMPRII多肽的异多聚体复合体及其用途)是可溶解的(例如BMPRII的胞外域)。在其它优选的实施方案中，用于本发明本公开内容的BMPRII多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，本公开内容的异多聚体复合体包含与SEQ ID NO:46、47、71、72、121、123、411或412的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种BMPRII多肽。在一些实施方案中，本公开内容的异多聚体复合体由与SEQ ID NO:46、47、71、72、121、123、411或412的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种BMPRII多肽组成或基本由其组成。

[0193] 在某些方面，本公开内容涉及包含MISRII多肽的蛋白质复合体。本文所用术语“MISRII”是指来自任何物种的缪勒抑制物质受体II型(MISRII)蛋白的家族和通过诱变或其它修饰衍生于所述MISRII蛋白的变体。本文对MISRII的提及要理解为是提及目前已鉴定形式的任一种。MISRII家族的成员一般是跨膜蛋白，由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0194] 术语“MISRII多肽”包括包含MISRII家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。所有本文所述MISRII相关多肽的氨基酸的编号以下面的人MISRII前体蛋白序列(SEQ ID NO:50)的编号为基础，除非另有特别标注。

[0195] 典型的人MISRII前体蛋白序列(NCBI Ref Seq NP_065434.1)如下：

[0196]

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

[0197] 信号肽用单下划线表示，胞外域用粗体字表示。

[0198] 加工的胞外MISRII多肽序列如下：

[0199]

PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGCRDSDEPGCESLHCDP SPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAPGESIWMAL(SEQ ID NO:51)

[0200] 编码MISRII前体蛋白的核酸序列见下(SEQ ID NO:52)，相当于Genbank参比序列NM_020547.2的核苷酸81-1799。信号序列用下划线表示。

[0201]

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAGGCGAACCTGTGTGTT
CTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAG
CTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAA
GGATGCCGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGG
CTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCTCCTCCAGGGAGCC
CTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCATCTGGATGGCACTGGTGCTGCTGGGGCTG
TTCCTCCTCCTCCTGCTGCTGCTGGGCAGCATCATCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGA
GCCAGTGCCAGAGCCAAGGCCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCT
CCCAGGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACTGGTTGCCATCAAGGCC
TTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTGTACGAACTTCCAGGCCTACAGCACGACCACAT
TGTCCGATTTATCACTGCCAGCCGGGGGGGTCCTGGCCGCCTGCTCTCTGGGCCCCTGCTGGTACTGGAACTGCATC
CCAAGGGCTCCCTGTGCCACTACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTG
GCCCAGGGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCACCGAGATCT
GAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACCTGGGCCTTGCCTTGGTGCTCCCTG
GCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCACAAGGCCCAGCTGCCATCATGGAAGCTGGCACCCAGAGG
TACATGGCACCAGAGCTCTTGGACAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTA
CTCTTTGGCTCTGCTCCTGTGGGAGATACTGAGCCGCTGCCCAGATTTGAGGCCTGACAGCAGTCCACCACCCTTCC
AACTGGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGCCTTGGCAGTGCAGGAGAGGAGG
CGTCCCTACATCCCATCCACCTGGCGCTGCTTTGCCACAGACCCTGATGGGCTGAGGGAGCTCCTAGAAGACTGTTG

GGATGCAGACCCAGAAGCACGGCTGACAGCTGAGTGTGTACAGCAGCGCCTGGCTGCCTTGGCCCATCCTCAAGAGA
GCCACCCCTTTCCAGAGAGCTGTCCACGTGGCTGCCCACCTCTCTGCCCAGAAGACTGTACTTCAATTCCTGCCCCT
ACCATCCTCCCCTGTAGGCCTCAGCGGAGTGCCTGCCACTTCAGCGTTCAGCAAGGCCCTTGTTCCAGGAATCCTCA
GCCTGCCTGTACCCTTTCTCCTGTG(SEQ ID NO:52)

[0202] 编码胞外人MISRII多肽的核酸序列如下：

[0203]

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGCTGCT
AGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAACCTGACCC
AAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCA
AGTCCCCGAGCCCACCCCAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTA
CAGCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCATCT
GGATGGCACTG(SEQ ID NO:53)

[0204] 人MISRII前体蛋白序列的可变同种型,同种型2(NCBI Ref Seq NP_001158162.1)如下：

[0205]

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

[0206] 信号肽用单下划线表示,胞外域用粗体字表示。

[0207] 加工的胞外MISRII多肽序列(同种型2)如下：

[0208]

PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGCRDSDEPGCESLHCDP
SPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAPGESIWMAL(SEQ ID NO:76)

[0209] 编码MISRII前体蛋白(同种型2)的核酸序列见下(SEQ ID NO:77),相当于Genbank参比序列NM_001164690.1的核苷酸81-1514。信号序列用下划线表示。

[0210]

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAGGCGAACCTGTGTGTT
CTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAG
CTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAA
GGATGCCGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGG
CTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCTCCTCCAGGGAGCC
CTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCATCTGGATGGCACTGGTGCTGCTGGGGCTG
TTCCTCCTCCTCCTGCTGCTGCTGGGCAGCATCATCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGA

GCCAGTGCCAGAGCCAAGGCCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCT
CCCAGGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACTGGTTGCCATCAAGGCC
TTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTGTACGAACTTCCAGGCCTACAGCACGACCACAT
TGTCCGATTTATCACTGCCAGCCGGGGGGGTCCTGGCCGCCTGCTCTCTGGGCCCCTGCTGGTACTGGAACTGCATC
CCAAGGGCTCCCTGTGCCACTACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTG
GCCCAGGGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCACCGAGATCT
GAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACCTGGGCCTTGCCTTGGTGCTCCCTG
GCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCACAAGGCCCAGCTGCCATCATGGAAGCTGGCACCCAGAGG
TACATGGCACCAGAGCTCTTGGACAAGACTCTGGACCTACAGGATTGGGGCATGGCCCTCCGACGAGCTGATATTTA
CTCTTTGGCTCTGCTCCTGTGGGAGATACTGAGCCGCTGCCCAGATTTGAGGCCTGCAGTCCACCACCCTTCCAACT
GGCCTATGAGGCAGAACTGGGCAATACCCCTACCTCTGATGAGCTATGGGCCTTGGCAGTGCAGGAGAGGAGGCGTC
CCTACATCCCATCCACCTGGCGCTGCTTTGCCACAGACCCTGATGGGC(SEQ ID NO:77)

[0211] 核酸序列编码加工的可溶性(胞外)人MISRII多肽(同种型2)如下：

[0212]

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGCTGCT
AGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAACCTGACCC
AAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCA
AGTCCCCGAGCCCACCCCAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTA
CAGCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCATCT
GGATGGCACTG(SEQ ID NO:78)

[0213] 人MISRII前体蛋白序列的可变同种型,同种型3(NCBI Ref Seq NP_001158163.1)如下：

[0214]

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

[0215] 信号肽用单下划线表示,胞外域用粗体字表示。

[0216] 加工的胞外MISRII多肽序列(同种型3)如下：

[0217]

PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGCRDSDEPGCESLHCDP
SPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAPGESIWMAL(SEQ ID NO:80)

[0218] 编码人MISRII前体蛋白(同种型3)的核酸序列见下(SEQ ID NO:81),相当于Genbank参比序列NM_001164691.1的核苷酸81-1514。信号序列用下划线表示。

[0219]

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAGGCGAACCTGTGTGTT
CTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGCTGCTAGATACAGGCACAGAGCTCCCCAGAG
CTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAA
GGATGCCGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCCCAGCCCTGG
CTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACAGCCATCTGCCTCCTCCAGGGAGCC
CTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCATCTGGATGGCACTGGTGCTGCTGGGGCTG
TTCCTCCTCCTCCTGCTGCTGCTGGGCAGCATCATCTTGGCCCTGCTACAGCGAAAGAACTACAGAGTGCGAGGTGA
GCCAGTGCCAGAGCCAAGGCCAGACTCAGGCAGGGACTGGAGTGTGGAGCTGCAGGAGCTGCCTGAGCTGTGTTTCT
CCCAGGTAATCCGGGAAGGAGGTCATGCAGTGGTTTGGGCCGGGCAGCTGCAAGGAAAACTGGTTGCCATCAAGGCC
TTCCCACCGAGGTCTGTGGCTCAGTTCCAAGCTGAGAGAGCATTGTACGAACTTCCAGGCCTACAGCACGACCACAT
TGTCCGATTTATCACTGCCAGCCGGGGGGGTCCTGGCCGCCTGCTCTCTGGGCCCCTGCTGGTACTGGAACTGCATC
CCAAGGGCTCCCTGTGCCACTACTTGACCCAGTACACCAGTGACTGGGGAAGTTCCCTGCGGATGGCACTGTCCCTG
GCCCAGGGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATATAAACCAGGTATTGCCCACCGAGATCT
GAGCAGCCAGAATGTGCTCATTCGGGAAGATGGATCGTGTGCCATTGGAGACCTGGGCCTTGCCTTGGTGCTCCCTG
GCCTCACTCAGCCCCCTGCCTGGACCCCTACTCAACCACAAGGCCCAGCTGCCATCATGGAAGACCCTGATGGGCTG
AGGGAGCTCCTAGAAGACTGTTGGGATGCAGACCCAGAAGCACGGCTGACAGCTGAGTGTGTACAGCAGCGCCTGGC
TGCCTTGGCCCATCCTCAAGAGAGCCACCCCTTTCCAGAGAGCTGTCCACGTGGCTGCCCACCTCTCTGCCCAGAAG
ACTGTACTTCAATTCCTGCCCCTACCATCCTCCCCTGTAGGCCTCAGCGGAGTGCCTGCCACTTCAGCGTTCAGCAA
GGCCCTTGTTCCAGGAATCCTCAGCCTGCCTGTACCCTTTCTCCTGTG(SEQ ID NO:81)

[0220] 编码加工的可溶性(胞外)人MISRII多肽(同种型3)的核酸序列如下：

[0221]

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGCTGCT
AGATACAGGCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAACCTGACCC
AAGACCGGGCACAGGTGGAAATGCAAGGATGCCGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCA
AGTCCCCGAGCCCACCCCAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTA
CAGCCATCTGCCTCCTCCAGGGAGCCCTGGGACTCCTGGCTCCCAGGGTCCCCAGGCTGCCCCAGGTGAGTCCATCT
GGATGGCACTG(SEQ ID NO:82)

[0222] 在某些实施方案中，本公开内容涉及包含至少一种MISRII多肽的异多聚体复合体，其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的MISRII多肽(例如包含MISRII多肽的异多聚体复合体及其用途)是可溶解的(例如MISRII的胞外域)。在其它优选的实施方案中，用于本发明本公开内容的MISRII多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，本公开内容的异多聚体复合体包含与SEQ ID NO:50、51、75、76、79或80的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种MISRII多肽。在一些实施方案中，本公开内容的异多聚体复合体由与SEQ ID NO:50、51、75、76、79或80的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种MISRII多肽组成或基本由其组成。

[0223] 在某些方面，本公开内容涉及包含ALK1多肽的蛋白质复合体。本文所用术语

“ALK1”是指来自任何物种的激活素受体样激酶-1蛋白的家族和通过诱变或其它修饰衍生于所述ALK1蛋白的变体。本文对ALK1的提及要理解为是提及目前已鉴定形式的任一种。ALK1家族的成员一般是跨膜蛋白，由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0224] 术语“ALK1多肽”包括包含ALK1家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。本文所述的所有ALK1相关多肽的氨基酸的编号以下面的人ALK1前体蛋白序列(SEQ ID NO:14)的编号为基础，除非另有特别标注。

[0225] 人ALK1前体蛋白序列(NCBI Ref Seq NP_000011.2)如下：

```
  1 MTLGSPRKGL LMLLMALVTQ GDPVKPSRGP LVTCTCESPH CKGPTCRGAW
 51 CTVVLVREEG RHPQEHRGCG NLHRELCRGR PTEFVNHYCC DSHLCNHNVS
101 LVLEATQPPS EQPGTDGQLALILGPVLALL ALVALGVLGL WHVRRRQEKQ
151 RGLHSELGES SLILKASEQG DSMLGDLLDSDCTTGSGSGL PFLVQRTVAR
201 QVALVECVGK GRYGEVWRGL WHGESVAVKI FSSRDEQSWF RETEIYNTVL
```

[0226]
```
251 LRHDNILGFI ASDMTSRNSS TQLWLITHYH EHGSLYDFLQ RQTLEPHLAL
301 RLAVSAACGL AHLHVEIFGT QGKPAIAHRD FKSRNVLVKS NLQCCIADLG
351 LAVMHSQGSD YLDIGNNPRV GTKRYMAPEV LDEQIRTDCF ESYKWTDIWA
401 FGLVLWEIAR RTIVNGIVED YRPPFYDVVP NDPSFEDMKK VVCVDQQTPT
451 IPNRLAADPV LSGLAQMMRE CWYPNPSARL TALRIKKTLQ KISNSPEKPK
501 VIQ (SEQ ID NO: 14)
```

[0227] 信号肽用单下划线表示，胞外域用粗体字表示。

[0228] 加工的胞外ALK1多肽序列如下：

[0229]

DPVKPSRGPLVTCTCESPHCKGPTCRGAWCTVVLVREEGRHPQEHRGCGNLHRELCRGRPTEFVNHYCCDSHLCNHNVSLVLEATQPPSEQPGTDGQ(SEQ ID NO:15)

[0230] 编码人ALK1前体蛋白的核酸序列见下(SEQ ID NO:16)，相当于Genbank参比序列NM_000020.2的核苷酸284-1792。信号序列用下划线表示。

[0231]

ATGACCTTGGGCTCCCCCAGGAAAGGCCTTCTGATGCTGCTGATGGCCTTGGTGACCCAGGGAGACCCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACCTGCACGTGTGAGAGCCCACATTGCAAGGGGCCTACCTGCCGGGGGGCCTGGTGCACAGTAGTGCTGGTGCGGGAGGAGGGGAGGCACCCCCAGGAACATCGGGGCTGCGGGAACTTGCACAGGGAGCTCTGCAGGGGGCGCCCCACCGAGTTCGTCAACCACTACTGCTGCGACAGCCACCTCTGCAACCACAACGTGTCCCTGGTGCTGGAGGCCACCCAACCTCCTTCGGAGCAGCCGGGAACAGATGGCCAGCTGGCCCTGATCCTGGGCCCCGTGCTGGCCTTGCTGGCCCTGGTGGCCCTGGGTGTCCTGGGCCTGTGGCATGTCCGACGGAGGCAGGAGAAGCAGCGTGGCCTGCACAGCGAGCTGGGAGAGTCCAGTCTCATCCTGAAAGCATCTGAGCAGGGCGACAGCATGTTGGGGGACCTCCTGGACAGTGACTGCACCACAGGGAGTGGCTCAGGGCTCCCCTTCCTGGTGCAGAGGACAGTGGCACGGCAGGTTGCCTTGGTGGAGTGTGTGGGAAAAGGCCGCTATGGCGAAGTGTGGCGGGGCTTGTGGCACGGTGAGAGTGTGGCCGTCAAGATCTTCTCCTCGAGGGATGAACAGTCCTGGTTCCGGGAGACTGAGATCTATAACACAGTGTTGCTCAGACACGACAACATCCTAGGCTTCATCGCCTCAGACATGACCTCCCGCAACTCGAGCACGCAGCTGTGGCTCATCACGCACTACCACGAGCACG

GCTCCCTCTACGACTTTCTGCAGAGACAGACGCTGGAGCCCCATCTGGCTCTGAGGCTAGCTGTGTCCGCGGCATGC
GGCCTGGCGCACCTGCACGTGGAGATCTTCGGTACACAGGGCAAACCAGCCATTGCCCACCGCGACTTCAAGAGCCG
CAATGTGCTGGTCAAGAGCAACCTGCAGTGTTGCATCGCCGACCTGGGCCTGGCTGTGATGCACTCACAGGGCAGCG
ATTACCTGGACATCGGCAACAACCCGAGAGTGGGCACCAAGCGGTACATGGCACCCGAGGTGCTGGACGAGCAGATC
CGCACGGACTGCTTTGAGTCCTACAAGTGGACTGACATCTGGGCCTTTGGCCTGGTGCTGTGGGAGATTGCCCGCCG
GACCATCGTGAATGGCATCGTGGAGGACTATAGACCACCCTTCTATGATGTGGTGCCCAATGACCCCAGCTTTGAGG
ACATGAAGAAGGTGGTGTGTGTGGATCAGCAGACCCCCACCATCCCTAACCGGCTGGCTGCAGACCCGGTCCTCTCA
GGCCTAGCTCAGATGATGCGGGAGTGCTGGTACCCAAACCCCTCTGCCCGACTCACCGCGCTGCGGATCAAGAAGAC
ACTACAAAAAATTAGCAACAGTCCAGAGAAGCCTAAAGTGATTCAA(SEQ ID NO:16)

[0232] 编码加工的胞外ALK1多肽的核酸序列如下：

[0233]

GACCCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACCTGCACGTGTGAGAGCCCACATTGCAAGGGGCCTACCTGCCG
GGGGGCCTGGTGCACAGTAGTGCTGGTGCGGGAGGAGGGGAGGCACCCCCAGGAACATCGGGGCTGCGGGAACTTGC
ACAGGGAGCTCTGCAGGGGGCGCCCCACCGAGTTCGTCAACCACTACTGCTGCGACAGCCACCTCTGCAACCACAAC
GTGTCCCTGGTGCTGGAGGCCACCCAACCTCCTTCGGAGCAGCCGGGAACAGATGGCCAG(SEQ ID NO:17)

[0234] 在某些实施方案中，本公开内容涉及包含至少一种ALK1多肽的异多聚体复合体，其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的ALK1多肽(例如包含ALK1多肽的异多聚体复合体及其用途)是可溶解的(例如ALK1的胞外域)。在其它优选的实施方案中，用于本公开内容发明的ALK1多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，本公开内容的异多聚体复合体包含与SEQ ID NO:14、15、124、126、413或414的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK1多肽。在一些实施方案中，本公开内容的异多聚体复合体由与SEQ ID NO:14、15、124、126、413或414的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK1多肽组成或基本由其组成。

[0235] 在某些方面，本公开内容涉及包含ALK2多肽的蛋白质复合体。本文所用术语“ALK2”是指自任何物种的激活素受体样激酶-2蛋白的家族来和通过诱变或其它修饰衍生于所述ALK2蛋白的变体。本文对ALK2的提及要理解为是提及目前已鉴定形式的任一种。ALK2家族的成员一般是跨膜蛋白，由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0236] 术语“ALK2多肽”包括包含ALK2家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。本文所述的所有ALK2相关多肽的氨基酸的编号以下面的人ALK2前体蛋白序列(SEQ ID NO:18)的编号为基础，除非另有特别标注。

[0237] 人ALK2前体蛋白序列(NCBIRefSeqNP_001096.1)如下：

[0238]

1 MVDGVMILPV LIMIALPSPSMEDEKPKVNP KLYMCVCEGL SCGNEDHCEG

51 QQCFSSLSINDGFHVYQKGC FQVYEQGKMT CKTPPSPGQA VECCQGDWCN

101 RNITAQLPTK GKSFPGTQNFHLEVGLIILS VVFAVCLLAC LLGVALRKFK

151 RRNQERLNPR DVEYGTIEGL ITTNVGDSTLADLLDHSCTS GSGSGLPFLV

201 QRTVARQITL LECVGKGRYG EVWRGSWQGE NVAVKIFSSRDEKSWFRETE

251 LYNTVMLRHE NILGFIASDM TSRHSSTQLW LITHYHEMGS LYDYLQLTTL

[0239] 301 DTVSCLRIVL SIASGLAHLH IEIFGTQGKP AIAHRDLKSK NILVKKNGQC

351 CIADLGLAVMHSQSTNQLDV GNNPRVGTKR YMAPEVLDET IQVDCFDSYK

401 RVDIWAFGLV LWEVARRMVSNGIVEDYKPP FYDVVPNDPS FEDMRKVVCV

451 DQQRPNIPNR WFSDPTLTSL AKLMKECWYQNPSARLTALR IKKTLTKIDN

501 SLDKLKTDC (SEQ ID NO: 18)

[0240] 信号肽用单下划线表示,胞外域用粗体字表示。

[0241] 加工的胞外ALK2多肽序列如下:

[0242]

MEDEKPKVNPKLYMCVCEGLSCGNEDHCEGQQCFSSLSINDGFHVYQKGCFQVYEQGKMTCKTPPSPGQAVECCQGD WCNRNITAQLPTKGKSFPGTQNFHLE(SEQ ID NO:19)

[0243] 编码人ALK2前体蛋白的核酸序列见下(SEQ ID NO:20),相当于Genbank参比序列NM_001105.4的核苷酸431-1957。信号序列用下划线表示。

[0244]

ATGGTAGATGGAGTGATGATTCTTCCTGTGCTTATCATGATTGCTCTCCCCTCCCCTAGTATGGAAGATGAGAAGCC CAAGGTCAACCCCAAACTCTACATGTGTGTGTGTGAAGGTCTCTCCTGCGGTAATGAGGACCACTGTGAAGGCCAGC AGTGCTTTTCCTCACTGAGCATCAACGATGGCTTCCACGTCTACCAGAAAGGCTGCTTCCAGGTTTATGAGCAGGGA AAGATGACCTGTAAGACCCCGCCGTCCCCTGGCCAAGCCGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACAT CACGGCCCAGCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAGGTTGGCCTCATTATTC TCTCTGTAGTGTTCGCAGTATGTCTTTTAGCCTGCCTGCTGGGAGTTGCTCTCCGAAAATTTAAAAGGCGCAACCAA GAACGCCTCAATCCCCGAGACGTGGAGTATGGCACTATCGAAGGGCTCATCACCACCAATGTTGGAGACAGCACTTT AGCAGATTTATTGGATCATTCGTGTACATCAGGAAGTGGCTCTGGTCTTCCTTTTCTGGTACAAAGAACAGTGGCTC GCCAGATTACACTGTTGGAGTGTGTCGGGAAAGGCAGGTATGGTGAGGTGTGGAGGGGCAGCTGGCAAGGGGAGAAT GTTGCCGTGAAGATCTTCTCCTCCCGTGATGAGAAGTCATGGTTCAGGGAAACGGAATTGTACAACACTGTGATGCT GAGGCATGAAAATATCTTAGGTTTCATTGCTTCAGACATGACATCAAGACACTCCAGTACCCAGCTGTGGTTAATTA CACATTATCATGAAATGGGATCGTTGTACGACTATCTTCAGCTTACTACTCTGGATACAGTTAGCTGCCTTCGAATA GTGCTGTCCATAGCTAGTGGTCTTGCACATTTGCACATAGAGATATTTGGGACCCAAGGGAAACCAGCCATTGCCCA TCGAGATTTAAAGAGCAAAAATATTCTGGTTAAGAAGAATGGACAGTGTTGCATAGCAGATTTGGGCCTGGCAGTCA TGCATTCCCAGAGCACCAATCAGCTTGATGTGGGGAACAATCCCCGTGTGGGCACCAAGCGCTACATGGCCCCCGAA GTTCTAGATGAAACCATCCAGGTGGATTGTTTCGATTCTTATAAAAGGGTCGATATTTGGGCCTTTGGACTTGTTTT GTGGGAAGTGGCCAGGCGGATGGTGAGCAATGGTATAGTGGAGGATTACAAGCCACCGTTCTACGATGTGGTTCCCA ATGACCCAAGTTTTGAAGATATGAGGAAGGTAGTCTGTGTGGATCAACAAAGGCCAAACATACCCAACAGATGGTTC TCAGACCCGACATTAACCTCTCTGGCCAAGCTAATGAAAGAATGCTGGTATCAAAATCCATCCGCAAGACTCACAGC ACTGCGTATCAAAAAGACTTTGACCAAAATTGATAATTCCCTCGACAAATTGAAAACTGACTGT(SEQ ID NO: 20)

[0245] 编码胞外ALK2多肽的核酸序列如下:

[0246]

ATGGAAGATGAGAAGCCCAAGGTCAACCCCAAACTCTACATGTGTGTGTGTGAAGGTCTCTCCTGCGGTAATGAGGACCACTGTGAAGGCCAGCAGTGCTTTTCCTCACTGAGCATCAACGATGGCTTCCACGTCTACCAGAAAGGCTGCTTCCAGGTTTATGAGCAGGGAAAGATGACCTGTAAGACCCCGCCGTCCCCTGGCCAAGCCGTGGAGTGCTGCCAAGGGGACTGGTGTAACAGGAACATCACGGCCCAGCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAG(SEQ ID NO:21)

[0247] 在某些实施方案中,本公开内容涉及包含至少一种ALK2多肽的异多聚体复合体,其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的ALK2多肽(例如包含ALK2多肽的异多聚体复合体及其用途)是可溶解的(例如ALK2的胞外域)。在其它优选的实施方案中,用于本公开内容发明的ALK2多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,本公开内容的异多聚体复合体包含与SEQ ID NO:18或19的氨基酸序列有至少70%、75%、80%、85%、90%、95%、97%、98%或99%同一性的至少一种ALK2多肽。在一些实施方案中,本公开内容的异多聚体复合体由与SEQ ID NO:18或19的氨基酸序列有至少70%、75%、80%、85%、90%、95%、97%、98%或99%同一性的至少一种ALK2多肽组成或基本由其组成。

[0248] 在某些方面,本公开内容涉及包含ALK3多肽的蛋白质复合体。本文所用术语“ALK3”是指来自任何物种的激活素受体样激酶-3蛋白的家族和通过诱变或其它修饰衍生于所述ALK3蛋白的变体。本文对ALK3的提及要理解为是提及目前已鉴定形式的任一种。ALK3家族的成员一般是跨膜蛋白,由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0249] 术语“ALK3多肽”包括包含ALK3家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。本文所述的所有ALK3相关多肽的氨基酸的编号以下面的人ALK3前体蛋白序列(SEQ ID NO:22)的编号为基础,除非另有特别标注。

[0250] 人ALK3前体蛋白序列(NCBIRefSeqNP_004320.2)如下:

[0251]

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

[0252] 信号肽用单下划线表示,胞外域用粗体字表示。

[0253] 加工的胞外ALK3多肽序列如下:

1 QNLDSMLHGT GMKSDSDQKK SENGVTLAPE DTLPFLKCYC SGHCPDDAIN NTCITNGHCF

[0254] 61 AIIEEDDQGE TTLASGCMKY EGSDFQCKDS PKAQLRRTIE CCRTNLCNQY LQPTLPPVVI

121 GPFFDGSIR(SEQ ID NO: 23)

[0255] 编码人ALK3前体蛋白的核酸序列见下(SEQ ID NO:24)，相当于Genbank参比序列NM_004329.2的核苷酸549-2144。信号序列用下划线表示，胞外域用粗体字表示。

1 ATGCCTCAGC TATACATTTA CATCAGATTA TTGGGAGCCT ATTTGTTCAT CATTTCTCGT

61 GTTCAAGGA**C AGAATCTGGA TAGTATGCTT CATGGCACTG GGATGAAATC AGACTCCGAC**

121 CAGAAAAAGT CAGAAAATGG AGTAACCTTA GCACCAGAGG ATACCTTGCC TTTTTTAAAG

181 TGCTATTGCT CAGGGCACTG TCCAGATGAT GCTATTAATA ACACATGCAT AACTAATGGA

241 CATTGCTTTG CCATCATAGA AGAAGATGAC CAGGGAGAAA CCACATTAGC TTCAGGGTGT

301 ATGAAATATG AAGGATCTGA TTTTCAGTGC AAAGATTCTC CAAAAGCCCA GCTACGCCGG

361 ACAATAGAAT GTTGTCGGAC CAATTTATGT AACCAGTATT TGCAACCCAC ACTGCCCCCT

421 GTTGTCATAG GTCCGTTTTT TGATGGCAGC ATTCGATGGC TGGTTTTGCT CATTTCTATG

481 GCTGTCTGCA TAATTGCTAT GATCATCTTC TCCAGCTGCT TTTGTTACAA ACATTATTGC

541 AAGAGCATCT CAAGCAGACG TCGTTACAAT CGTGATTTGG AACAGGATGA AGCATTTATT

601 CCAGTTGGAG AATCACTAAA AGACCTTATT GACCAGTCAC AAAGTTCTGG TAGTGGGTCT

661 GGACTACCTT TATTGGTTCA GCGAACTATT GCCAAACAGA TTCAGATGGT CCGGCAAGTT

721 GGTAAAGGCC GATATGGAGA AGTATGGATG GGCAAATGGC GTGGCGAAAA AGTGGCGGTG

[0256] 781 AAAGTATTCT TTACCACTGA AGAAGCCAGC TGGTTTCGAG AAACAGAAAT CTACCAAACT

841 GTGCTAATGC GCCATGAAAA CATACTTGGT TTCATAGCGG CAGACATTAA AGGTACAGGT

901 TCCTGGACTC AGCTCTATTT GATTACTGAT TACCATGAAA ATGGATCTCT CTATGACTTC

961 CTGAAATGTG CTACACTGGA CACCAGAGCC CTGCTTAAAT TGGCTTATTC AGCTGCCTGT

1021 GGTCTGTGCC ACCTGCACAC AGAAATTTAT GGCACCCAAG GAAAGCCCGC AATTGCTCAT

1081 CGAGACCTAA AGAGCAAAAA CATCCTCATC AAGAAAAATG GGAGTTGCTG CATTGCTGAC

1141 CTGGGCCTTG CTGTTAAATT CAACAGTGAC ACAAATGAAG TTGATGTGCC CTTGAATACC

1201 AGGGTGGGCA CCAAACGCTA CATGGCTCCC GAAGTGCTGG ACGAAAGCCT GAACAAAAAC

1261 CACTTCCAGC CCTACATCAT GGCTGACATC TACAGCTTCG GCCTAATCAT TTGGGAGATG

1321 GCTCGTCGTT GTATCACAGG AGGGATCGTG GAAGAATACC AATTGCCATA TTACAACATG

1381 GTACCGAGTG ATCCGTCATA CGAAGATATG CGTGAGGTTG TGTGTGTCAA ACGTTTGCGG

1441 CCAATTGTGT CTAATCGGTG GAACAGTGAT GAATGTCTAC GAGCAGTTTT GAAGCTAATG

1501 TCAGAATGCT GGGCCCACAA TCCAGCCTCC AGACTCACAG CATTGAGAAT TAAGAAGACG

1561 CTTGCCAAGA TGGTTGAATC CCAAGATGTA AAAATC (SEQ ID NO: 24)

[0257] 编码胞外人ALK3多肽的核酸序列如下：

[0258] 1 CAGAATCTGG ATAGTATGCT TCATGGCACT GGGATGAAAT CAGACTCCGA CCAGAAAAAG

[0259]

```
 61  TCAGAAAATG GAGTAACCTT AGCACCAGAG GATACCTTGC CTTTTTTAAA GTGCTATTGC
121  TCAGGGCACT GTCCAGATGA TGCTATTAAT AACACATGCA TAACTAATGG ACATTGCTTT
181  GCCATCATAG AAGAAGATGA CCAGGGAGAA ACCACATTAG CTTCAGGGTG TATGAAATAT
241  GAAGGATCTG ATTTTCAGTG CAAAGATTCT CCAAAAGCCC AGCTACGCCG GACAATAGAA
301  TGTTGTCGGA CCAATTTATG TAACCAGTAT TTGCAACCCA CACTGCCCCC TGTTGTCATA
361  GGTCCGTTTT TTGATGGCAG CATTCGA  (SEQ ID NO: 25)
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[0260] 有活性的(例如配体结合)ALK3多肽的通式是包含始于SEQ ID NO:22的任何氨基酸位置25-31(即25、26、27、28、29、30或31位)和止于SEQ ID NO:22的任何氨基酸位置140-152(即140、141、142、143、144、145、146、147、148、149、150、151或152)的多肽的通式。参见美国专利8,338,377,其教导内容通过引用以其整体结合到本文中。

[0261] 在某些实施方案中,本公开内容涉及包含至少一种ALK3多肽的异多聚体复合体,其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的ALK3多肽(例如包含ALK3多肽的异多聚体复合体及其用途)是可溶解的(例如ALK3的胞外域)。在其它优选的实施方案中,用于本公开内容发明的ALK3多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,本公开内容的异多聚体复合体包含至少一种ALK3多肽,其包含始于SEQ ID NO:22的任何氨基酸25-31位(即25、26、27、28、29、30或31位)和止于SEQ ID NO:22的任何氨基酸140-153位(即140、141、142、143、144、145、146、147、148、149、150、151或152)的氨基酸、由其组成或基本由其组成。在一些实施方案中,本公开内容的异多聚体复合体包含与SEQ ID NO:22、23、115、117、407或408的氨基酸序列有至少70%、75%、80%、85%、90%、95%、97%、98%或99%同一性的至少一种ALK3多肽。在一些实施方案中,本公开内容的异多聚体复合体由与SEQ ID NO:22、23、115、117、407或408的氨基酸序列有至少70%、75%、80%、85%、90%、95%、97%、98%或99%同一性的至少一种ALK3多肽组成或基本由其组成。

[0262] 在某些方面,本公开内容涉及包含ALK4多肽的蛋白质复合体。本文所用术语“ALK4”是指来自任何物种的激活素受体样激酶-4蛋白的家族和通过诱变或其它修饰衍生于所述ALK4蛋白的变体。本文对ALK4的提及要理解为是提及目前已鉴定形式的任一种。ALK4家族的成员一般是跨膜蛋白,由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0263] 术语“ALK4多肽”包括包含ALK4家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。本文所述的所有ALK4相关多肽的氨基酸的编号以下面的人ALK4前体蛋白序列(SEQ ID NO:26)的编号为基础,除非另有特别标注。

[0264] 典型的人ALK4前体蛋白序列(NCBI Ref Seq NP_004293)如下:

1 MAESAGASSF FPLVVLLLAG SGGSGPRGVQ ALLCACTSCL QANYTCETDG ACMVSIFNLD
61 GMEHHVRTCI PKVELVPAGK PFYCLSSEDL RNTHCCYTDY CNRIDLRVPS GHLKEPEHPS
121 MWGPVELVGI IAGPVFLLFL IIIIVFLVIN YHQRVYHNRQ RLDMEDPSCE MCLSKDKTLQ
181 DLVYDLSTSG SGSGLPLFVQ RTVARTIVLQ EIIGKGRFGE VWRGRWRGGD VAVKIFSSRE
[0265] 241 ERSWFREAEI YQTVMLRHEN ILGFIAADNK DNGTWTQLWL VSDYHEHGSL FDYLNRYTVT
301 IEGMIKLALS AASGLAHLHM EIVGTQGKPG IAHRDLKSKN ILVKKNGMCA IADLGLAVRH
361 DAVTDTIDIA PNQRVGTKRY MAPEVLDETI NMKHFDSFKC ADIYALGLVY WEIARRCNSG
421 GVHEEYQLPY YDLVPSDPSI EEMRKVVCDQ KLRPNIPNWW QSYEALRVMG KMMRECWYAN
481 GAARLTALRI KKTLSQLSVQ EDVKI (SEQ ID NO: 26)

[0266] 信号肽用单下划线表示，胞外域用粗体字表示。

[0267] 加工的胞外人ALK4多肽序列如下：

[0268]

SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGKPFYCLSSEDLRNTHCCYTDY CNRIDLRVPSGHLKEPEHPSMWGPVE(SEQ ID NO:27)

[0269] 编码ALK4前体蛋白的核酸序列见下(SEQ ID NO:28)，相当于Genbank参比序列NM_004302.4的核苷酸78-1592。信号序列用下划线表示，胞外域用粗体字表示。

[0270]

ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGCCGGCAGCGGCGGGTCCGGGCC CCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTACACGTGTGAGACAGATGGGGCCT GCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACCATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCT GCCGGGAAGCCCTTCTACTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAG GATCGACTTGAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAGCTGGTAG GCATCATCGCCGGCCCGGTGTTCCTCCTGTTCCTCATCATCATCATTGTTTTCCTTGTCATTAACTATCATCAGCGT GTCTATCACAACCGCCAGAGACTGGACATGGAAGATCCCTCATGTGAGATGTGTCTCTCCAAAGACAAGACGCTCCA GGATCTTGTCTACGATCTCTCCACCTCAGGGTCTGGCTCAGGGTTACCCCTCTTTGTCCAGCGCACAGTGGCCCGAA CCATCGTTTTACAAGAGATTATTGGCAAGGGTCGGTTTGGGGAAGTATGGCGGGGCCGCTGGAGGGGTGGTGATGTG GCTGTGAAAATATTCTCTTCTCGTGAAGAACGGTCTTGGTTCAGGGAAGCAGAGATATACCAGACGGTCATGCTGCG CCATGAAAACATCCTTGGATTTATTGCTGCTGACAATAAAGATAATGGCACCTGGACACAGCTGTGGCTTGTTTCTG ACTATCATGAGCACGGGTCCCTGTTTGATTATCTGAACCGGTACACAGTGACAATTGAGGGGATGATTAAGCTGGCC TTGTCTGCTGCTAGTGGGCTGGCACACCTGCACATGGAGATCGTGGGCACCCAAGGGAAGCCTGGAATTGCTCATCG AGACTTAAAGTCAAAGAACATTCTGGTGAAGAAAAATGGCATGTGTGCCATAGCAGACCTGGGCCTGGCTGTCCGTC ATGATGCAGTCACTGACACCATTGACATTGCCCCGAATCAGAGGGTGGGGACCAAACGATACATGGCCCCTGAAGTA CTTGATGAAACCATTAATATGAAACACTTTGACTCCTTTAAATGTGCTGATATTTATGCCCTCGGGCTTGTATATTG GGAGATTGCTCGAAGATGCAATTCTGGAGGAGTCCATGAAGAATATCAGCTGCCATATTACGACTTAGTGCCCTCTG ACCCTTCCATTGAGGAAATGCGAAAGGTTGTATGTGATCAGAAGCTGCGTCCCAACATCCCCAACTGGTGGCAGAGT TATGAGGCACTGCGGGTGATGGGGAAGATGATGCGAGAGTGTTGGTATGCCAACGGCGCAGCCCGCCTGACGGCCCT GCGCATCAAGAAGACCCTCTCCCAGCTCAGCGTGCAGGAAGACGTGAAGATC(SEQ ID NO:28)

[0271] 编码胞外ALK4多肽的核酸序列如下：

[0272]

TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTACACGTGTGAGACAGA
TGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACCATGTGCGCACCTGCATCCCCAAAGTGGAGC
TGGTCCCTGCCGGGAAGCCCTTCTACTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTAC
TGCAACAGGATCGACTTGAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGA
G(SEQ ID NO:29)

[0273] 人ALK4前体蛋白序列的的可变同种型,同种型C(NCBI Ref Seq NP_064733.3)如下:

[0274]

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

[0275] 信号肽用单下划线表示,胞外域用粗体字表示。

[0276] 加工的胞外ALK4多肽序列(同种型C)如下:

[0277]

SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGKPFYCLSSEDLRNTHCCYTDY
CNRIDLRVPSGHLKEPEHPSMWGPVE(SEQ ID NO:84)

[0278] 编码ALK4前体蛋白(同种型C)的核酸序列见下(SEQ ID NO:85),相当于Genbank参比序列NM_020328.3的核苷酸78-1715。信号序列用下划线表示,胞外域用粗体字表示。

[0279]

ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGCCGGCAGCGGCGGGTCCGGGCC
CCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTACACGTGTGAGACAGATGGGGCCT
GCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACCATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCT
GCCGGGAAGCCCTTCTACTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAG
GATCGACTTGAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAGCTGGTAG
GCATCATCGCCGGCCCGGTGTTCCTCCTGTTCCTCATCATCATCATTGTTTTCCTTGTCATTAACTATCATCAGCGT
GTCTATCACAACCGCCAGAGACTGGACATGGAAGATCCCTCATGTGAGATGTGTCTCTCCAAAGACAAGACGCTCCA
GGATCTTGTCTACGATCTCTCCACCTCAGGGTCTGGCTCAGGGTTACCCCTCTTTGTCCAGCGCACAGTGGCCCGAA
CCATCGTTTTACAAGAGATTATTGGCAAGGGTCGGTTTGGGGAAGTATGGCGGGGCCGCTGGAGGGGTGGTGATGTG
GCTGTGAAAATATTCTCTTCTCGTGAAGAACGGTCTTGGTTCAGGGAAGCAGAGATATACCAGACGGTCATGCTGCG
CCATGAAAACATCCTTGGATTTATTGCTGCTGACAATAAAGCAGACTGCTCATTCCTCACATTGCCATGGGAAGTTG
TAATGGTCTCTGCTGCCCCCAAGCTGAGGAGCCTTAGACTCCAATACAAGGGAGGAAGGGGAAGAGCAAGATTTTTA
TTCCCACTGAATAATGGCACCTGGACACAGCTGTGGCTTGTTTCTGACTATCATGAGCACGGGTCCCTGTTTGATTA

TCTGAACCGGTACACAGTGACAATTGAGGGGATGATTAAGCTGGCCTTGTCTGCTGCTAGTGGGCTGGCACACCTGC
ACATGGAGATCGTGGGCACCCAAGGGAAGCCTGGAATTGCTCATCGAGACTTAAAGTCAAAGAACATTCTGGTGAAG
AAAAATGGCATGTGTGCCATAGCAGACCTGGGCCTGGCTGTCCGTCATGATGCAGTCACTGACACCATTGACATTGC
CCCGAATCAGAGGGTGGGGACCAAACGATACATGGCCCCTGAAGTACTTGATGAAACCATTAATATGAAACACTTTG
ACTCCTTTAAATGTGCTGATATTTATGCCCTCGGGCTTGTATATTGGGAGATTGCTCGAAGATGCAATTCTGGAGGA
GTCCATGAAGAATATCAGCTGCCATATTACGACTTAGTGCCCTCTGACCCTTCCATTGAGGAAATGCGAAAGGTTGT
ATGTGATCAGAAGCTGCGTCCCAACATCCCCAACTGGTGGCAGAGTTATGAGGCACTGCGGGTGATGGGGAAGATGA
TGCGAGAGTGTTGGTATGCCAACGGCGCAGCCCGCCTGACGGCCCTGCGCATCAAGAAGACCCTCTCCCAGCTCAGC
GTGCAGGAAGACGTGAAGATC(SEQ ID NO:85)

[0280] 编码胞外ALK4多肽(同种型C)的核酸序列如下：

[0281] TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTACACGTGT
GAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACCATGTGCGCACCTGCATCCCCAA
AGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTACTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACA
CTGACTACTGCAACAGGATCGACTTGAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGC
CCGGTGGAG

[0282] (SEQ ID NO:86)

[0283] 在某些实施方案中，本公开内容涉及包含至少一种ALK4多肽的异多聚体复合体，其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的ALK4多肽(例如包含ALK4多肽的异多聚体复合体及其用途)是可溶解的(例如ALK4的胞外域)。在其它优选的实施方案中，用于本公开内容发明的ALK4多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，本公开内容的异多聚体复合体包含与SEQ ID NO:26、27、83、84、104、106、403或404的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK4多肽。在一些实施方案中，本公开内容的异多聚体复合体由与SEQ ID NO:26、27、83、84、104、106、403或404的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK4多肽组成或基本由其组成。

[0284] 在某些方面，本公开内容涉及包含ALK5多肽的蛋白质复合体。本文所用术语“ALK5”是指来自任何物种的激活素受体样激酶-5蛋白的家族和通过诱变或其它修饰衍生于所述ALK4蛋白的变体。本文对ALK5的提及要理解为是提及目前已鉴定形式的任一种。ALK5家族的成员一般是跨膜蛋白，由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0285] 术语“ALK5多肽”包括包含ALK5家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。本文所述的所有ALK5相关多肽的氨基酸的编号以下面的人ALK5前体蛋白序列(SEQ ID NO:30)的编号为基础，除非另有特别标注。

[0286] 典型的人ALK5前体蛋白序列(NCBI Ref Seq NP_004603.1)如下：

1 MEAAVAAPRP RLLLLVLAAA AAAA**AALLPG ATALQCFCHL CTKDNFTCVT DGLCFVSVTE**

61 TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE LPTTVKSSPG

121 LGPVELAAVI AGPVCFVCIS LMLMVYICHN RTVIHHRVPN EEDPSLDRPF ISEGTTLKDL

181 IYDMTTSGSG SGLPLLVQRT IARTIVLQES IGKGRFGEVW RGKWRGEEVA VKIFSSREER

[0287] 241 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS DYHEHGSLFD YLNRYTVTVE

301 GMIKLALSTA SGLAHLHMEI VGTQGKPAIA HRDLKSKNIL VKKNGTCCIA DLGLAVRHDS

361 ATDTIDIAPN HRVGTKRYMA PEVLDDSINM KHFESFKRAD IYAMGLVFWE IARRCSIGGI

421 HEDYQLPYYD LVPSDPSVEE MRKVVCEQKL RPNIPNRWQS CEALRVMAKI MRECWYANGA

481 ARLTALRIKK TLSQLSQQEG IKM (SEQ ID NO: 30)

[0288] 信号肽用单下划线表示,胞外域用粗体字表示。

[0289] 加工的胞外ALK5多肽序列如下:

[0290]

AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVCAPSSKTGSVTTTYCC NQDHCNKIELPTTVKSSPGLGPVEL(SEQ ID NO:31)

[0291] 编码ALK5前体蛋白的核酸序列见下(SEQ ID NO:32),相当于Genbank参比序列NM_004612.2的核苷酸77-1585。信号序列用下划线表示,胞外域用粗体字表示。

[0292]

ATGGAGGCGGCGGTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGCGGCGGCGGCGGCGGC GCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAATTTTACTTGTGTGACAGATG GGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTA ATTCCTCGAGATAGGCCGTTTGTATGTGCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCA GGACCATTGCAATAAAATAGAACTTCCAACTACTGTAAAGTCATCACCTGGCCTTGGTCCTGTGGAACTGGCAGCTG TCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACTCATGTTGATGGTCTATATCTGCCACAACCGCACTGTCATT CACCATCGAGTGCCAAATGAAGAGGACCCTTCATTAGATCGCCCTTTTATTTCAGAGGGTACTACGTTGAAAGACTT AATTTATGATATGACAACGTCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAACAATTGCGAGAACTATTG TGTTACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGGGGAGAAGAAGTTGCTGTT AAGATATTCTCCTCTAGAGAAGAACGTTCGTGGTTCCGTGAGGCAGAGATTTATCAAACTGTAATGTTACGTCATGA AAACATCCTGGGATTTATAGCAGCAGACAATAAAGACAATGGTACTTGGACTCAGCTCTGGTTGGTGTCAGATTATC ATGAGCATGGATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAAACTTGCTCTGTCC ACGGCGAGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGAAAGCCAGCCATTGCTCATAGAGATTT GAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACTTGCTGTATTGCAGACTTAGGACTGGCAGTAAGACATGATT CAGCCACAGATACCATTGATATTGCTCCAAACCACAGAGTGGGAACAAAAAGGTACATGGCCCCTGAAGTTCTCGAT GATTCCATAAATATGAAACATTTTGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCTGGGAAAT TGCTCGACGATGTTCCATTGGTGGAATTCATGAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCTGACCCAT CAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAGGCCAAATATCCCAAACAGATGGCAGAGCTGTGAA GCCTTGAGAGTAATGGCTAAAATTATGAGAGAATGTTGGTATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGAT TAAGAAAACATTATCGCAACTCAGTCAACAGGAAGGCATCAAAATG(SEQ ID NO:32)

[0293] 编码胞外人ALK5多肽的核酸序列如下:

[0294]

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAATTTTACTTGTGTGAC AGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTG ACTTAATTCCTCGAGATAGGCCGTTTGTATGTGCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGC AATCAGGACCATTGCAATAAAATAGAACTTCCAACTACTGTAAAGTCATCACCTGGCCTTGGTCCTGTGGAACTG (SEQ ID NO:33)

[0295] 人ALK5前体蛋白序列的可变同种型,同种型2(NCBI Ref Seq XP_005252207.1)如下:

1 MEAAVAAPRP RLLLLVLAAA AAAA**AALLPG ATALQCFCHL CTKDNFTCVT DGLCFVSVTE**
61 **TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE LPTTGPFSVK**
121 **SSPGLGPVEL** AAVIAGPVCF VCISLMLMVY ICHNRTVIHH RVPNEEDPSL DRPFISEGTT
181 LKDLIYDMTT SGSGSGLPLL VQRTIARTIV LQESIGKGRF GEVWRGKWRG EEVAVKIFSS

[0296] 241 REERSWFREA EIYQTVMLRH ENILGFIAAD NKDNGTWTQL WLVSDYHEHG SLFDYLNRYT
301 VTVEGMIKLA LSTASGLAHL HMEIVGTQGK PAIAHRDLKS KNILVKKNGT CCIADLGLAV
361 RHDSATDTID IAPNHRVGTK RYMAPEVLDD SINMKHFESF KRADIYAMGL VFWEIARRCS
421 IGGIHEDYQL PYYDLVPSDP SVEEMRKVVC EQKLRPNIPN RWQSCEALRV MAKIMRECWY
481 ANGAARLTAL RIKKTLSQLS QQEGIKM (SEQ ID NO: 87)

[0297] 信号肽用单下划线表示,胞外域用粗体字表示。

[0298] 加工的胞外ALK5多肽序列(同种型2)如下:

[0299]

AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVCAPSSKTGSVTTTYCC NQDHCNKIELPTTGPFSVKSSPGLGPVEL(SEQ ID NO:88)

[0300] 编码人ALK5前体蛋白(同种型2)的核酸序列见下(SEQ ID NO:89),相当于Genbank参比序列XM_005252150.1的核苷酸77-1597。信号序列用下划线表示,胞外域用粗体字表示。

[0301]

ATGGAGGCGGCGGTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGCGGCGGCGGCG
GCGGCGCTGCTCCCGG
GGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAG
ACAATTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGT
CACAGAGACCACAGACAAAGTTATACACAACAGCATGTGTAT
AGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
GCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCT
GCAATCAGGACCATTGCAATAAAATAGAACTTCCAACTACTG
GCCCTTTTTCAGTAAAGTCATCACCTGGCCTTGGTCCTGTGGA
ACTG
GCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACTCATGTTGATGGTCTATATCTGCCACAACCGCAC TGTCATTCACCATCGAGTGCCAAATGAAGAGGACCCTTCATTAGATCGCCCTTTTATTTCAGAGGGTACTACGTTGA

AAGACTTAATTTATGATATGACAACGTCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAACAATTGCGAGA
ACTATTGTGTTACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGGGGAGAAGAAGT
TGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTCGTGGTTCCGTGAGGCAGAGATTTATCAAACTGTAATGTTAC
GTCATGAAAACATCCTGGGATTTATAGCAGCAGACAATAAAGACAATGGTACTTGGACTCAGCTCTGGTTGGTGTCA
GATTATCATGAGCATGGATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAAACTTGC
TCTGTCCACGGCGAGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGAAAGCCAGCCATTGCTCATA
GAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACTTGCTGTATTGCAGACTTAGGACTGGCAGTAAGA
CATGATTCAGCCACAGATACCATTGATATTGCTCCAAACCACAGAGTGGGAACAAAAAGGTACATGGCCCCTGAAGT
TCTCGATGATTCCATAAATATGAAACATTTTGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCT
GGGAAATTGCTCGACGATGTTCCATTGGTGGAATTCATGAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCT
GACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAGGCCAAATATCCCAAACAGATGGCAGAG
CTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGAGAGAATGTTGGTATGCCAATGGAGCAGCTAGGCTTACAGCAT
TGCGGATTAAGAAAACATTATCGCAACTCAGTCAACAGGAAGGCATCAAAATG(SEQ ID NO:89)

[0302] 编码加工的胞外ALK5多肽的核酸序列如下：

[0303]

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAATTTTACTTGTGTGAC
AGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTG
ACTTAATTCCTCGAGATAGGCCGTTTGTATGTGCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGC
AATCAGGACCATTGCAATAAAATAGAACTTCCAACTACTGGCCCTTTTTCAGTAAAGTCATCACCTGGCCTTGGTCC
TGTGGAACTG(SEQ ID NO:90)

[0304] 在某些实施方案中，本公开内容涉及包含至少一种ALK5多肽的异多聚体复合体，其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的ALK5多肽(例如包含ALK5多肽的异多聚体复合体及其用途)是可溶解的(例如ALK5的胞外域)。在其它优选的实施方案中，用于本公开内容发明的ALK5多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，本公开内容的异多聚体复合体包含与SEQ ID NO:30、31、87或88的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK5多肽。在一些实施方案中，本公开内容的异多聚体复合体由与SEQ ID NO:30、31、87或88的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK5多肽组成或基本由其组成。

[0305] 在某些方面，本公开内容涉及包含ALK6多肽的蛋白质复合体。本文所用术语“ALK6”是指来自任何物种的激活素受体样激酶-6蛋白的家族和通过诱变或其它修饰衍生于所述ALK6蛋白的变体。本文对ALK6的提及要理解为是提及目前已鉴定形式的任一种。ALK6家族的成员一般是跨膜蛋白，由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0306] 术语“ALK6多肽”包括包含ALK6家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。本文所述的所有ALK6相关多肽的氨基酸的编号以下面的人ALK6前体蛋白序列(SEQ ID NO:34)的编号为基础，除非另有特别标注。

[0307] 典型的人ALK6前体蛋白序列(NCBI Ref Seq NP_001194.1)如下：

1 MLLRSAGKLN VGT**KKEDGES TAPTPRPKVL RCKCHHHCPE DSVNNICSTD GYCFTMIEED**

61 **DSGLPVVTSG CLGLEGSDFQ CRDTPIPHQR RSIECCTERN ECNKDLHPTL PPLKNRDFVD**

121 **GPIHHR**ALLI SVTVCSLLLV LIILFCYFRY KRQETRPRYS IGLEQDETYI PPGESLRDLI

181 EQSQSSGSGS GLPLLVQRTI AKQIQMVKQI GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS

[0308] 241 WFRETEIYQT VLMRHENILG FIAADIKGTG SWTQLYLITD YHENGSLYDY LKSTTLDAKS

301 MLKLAYSSVS GLCHLHTEIF STQGKPAIAH RDLKSKNILV KKNGTCCIAD LGLAVKFISD

361 TNEVDIPPNT RVGTKRYMPP EVLDESLNRN HFQSYIMADM YSFGLILWEV ARRCVSGGIV

421 EEYQLPYHDL VPSDPSYEDM REIVCIKKLR PSFPNRWSSD ECLRQMGKLM TECWAHNPAS

481 RLTALRVKKT LAKMSESQDI KL (SEQ ID NO: 34)

[0309] 信号肽用单下划线表示，胞外域用粗体字表示。

[0310] 加工的胞外ALK6多肽序列如下：

[0311]

KKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMIEEDDSGLPVVTSGCLGLEGSDFQCRDTPIPHQR RSIECCTERNECNKDLHPTLPPLKNRDFVDGPIHHR(SEQ ID NO:35)

[0312] 编码ALK6前体蛋白的核酸序列见下(SEQ ID NO:36)，相当于Genbank参比序列NM_001203.2的核苷酸275-1780。信号序列用下划线表示，胞外域用粗体字表示。

[0313]

ATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACCAAGAAAGAGGATGGTGAGAGTACAGCCCCCACCCCCCG TCCAAAGGTCTTGCGTTGTAAATGCCACCACCATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGAT ATTGTTTCACGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAAGGCTCA GATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAAGGAACGAATGTAA TAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTTGATGGACCTATACACCACAGGGCTTTAC TTATATCTGTGACTGTCTGTAGTTTGCTCTTGGTCCTTATCATATTATTTTGTTACTTCCGGTATAAAAGACAAGAA ACCAGACCTCGATACAGCATTGGGTTAGAACAGGATGAAACTTACATTCCTCCTGGAGAATCCCTGAGAGACTTAAT TGAGCAGTCTCAGAGCTCAGGAAGTGGATCAGGCCTCCCTCTGCTGGTCCAAAGGACTATAGCTAAGCAGATTCAGA TGGTGAAACAGATTGGAAAAGGTCGCTATGGGGAAGTTTGGATGGGAAAGTGGCGTGGCGAAAAGGTAGCTGTGAAA GTGTTCTTCACCACAGAGGAAGCCAGCTGGTTCAGAGAGACAGAAATATATCAGACAGTGTTGATGAGGCATGAAAA CATTTTGGGTTTCATTGCTGCAGATATCAAAGGGACAGGGTCCTGGACCCAGTTGTACCTAATCACAGACTATCATG AAAATGGTTCCCTTTATGATTATCTGAAGTCCACCACCCTAGACGCTAAATCAATGCTGAAGTTAGCCTACTCTTCT GTCAGTGGCTTATGTCATTTACACACAGAAATCTTTAGTACTCAAGGCAAACCAGCAATTGCCCATCGAGATCTGAA AAGTAAAAACATTCTGGTGAAGAAAAATGGAACTTGCTGTATTGCTGACCTGGGCCTGGCTGTTAAATTTATTAGTG ATACAAATGAAGTTGACATACCACCTAACACTCGAGTTGGCACCAAACGCTATATGCCTCCAGAAGTGTTGGACGAG AGCTTGAACAGAAATCACTTCCAGTCTTACATCATGGCTGACATGTATAGTTTTGGCCTCATCCTTTGGGAGGTTGC TAGGAGATGTGTATCAGGAGGTATAGTGGAAGAATACCAGCTTCCTTATCATGACCTAGTGCCCAGTGACCCCTCTT ATGAGGACATGAGGGAGATTGTGTGCATCAAGAAGTTACGCCCCTCATTCCCAAACCGGTGGAGCAGTGATGAGTGT CTAAGGCAGATGGGAAAACTCATGACAGAATGCTGGGCTCACAATCCTGCATCAAGGCTGACAGCCCTGCGGGTTAA GAAAACACTTGCCAAAATGTCAGAGTCCCAGGACATTAAACTC(SEQ ID NO:36)

[0314] 编码加工的胞外ALK6多肽的核酸序列如下：

[0315]

AAGAAAGAGGATGGTGAGAGTACAGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATGCCACCACCATTGTCC
AGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCACGATGATAGAAGAGGATGACTCTGGGTTGC
CTGTGGTCACTTCTGGTTGCCTAGGACTAGAAGGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGA
AGATCAATTGAATGCTGCACAGAAAGGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAG
AGATTTTGTTGATGGACCTATACACCACAGG(SEQ ID NO:37)

[0316] 人ALK6前体蛋白序列的可变同种型,同种型2(NCBI Ref Seq NP_001243722.1)如下:

1 MGWLEELNWQ LHIFLLILLS MHTRA**NFLDN MLLRSAGKLN VGTKKEDGES TAPTPRPKVL**
61 **RCKCHHHCPE DSVNNICSTD GYCFTMIEED DSGLPVVTSG CLGLEGSDFQ CRDTPIPHQR**
121 **RSIECCTERN ECNKDLHPTL PPLKNRDFVD GPIHHR**ALLI SVTVCSLLLV LIILFCYFRY
181 KRQETRPRYS IGLEQDETYI PPGESLRDLI EQSQSSGSGS GLPLLVQRTI AKQIQMVKQI

[0317] 241 GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT VLMRHENILG FIAADIKGTG
301 SWTQLYLITD YHENGSLYDY LKSTTLDAKS MLKLAYSSVS GLCHLHTEIF STQGKPAIAH
361 RDLKSKNILV KKNGTCCIAD LGLAVKFISD TNEVDIPPNT RVGTKRYMPP EVLDESLNRN
421 HFQSYIMADM YSFGLILWEV ARRCVSGGIV EEYQLPYHDL VPSDPSYEDM REIVCIKKLR
481 PSFPNRWSSD ECLRQMGKLM TECWAHNPAS RLTALRVKKT LAKMSESQDI KL (SEQ ID NO: 91)

[0318] 信号肽用单下划线表示,胞外域用粗体字表示。

[0319] 加工的胞外ALK6多肽序列(同种型2)如下:

[0320]

NFLDNMLLRSAGKLNVGTKKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMIEEDDSGLPVVTSGCL
GLEGSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFVDGPIHHR(SEQ ID NO:92)

[0321] 编码人ALK6前体蛋白(同种型2)的核酸序列见下,相当于Genbank参比序列NM_001256793.1的核苷酸22-1617。信号序列用下划线表示,胞外域用粗体字表示。

[0322]

ATGGGTTGGCTGGAAGAACTAAACTGGCAGCTTCACATTTTCTTGCTCATTCTTCTCTCTATGCACACAAGGGCAAA
CTTCCTTGATAACATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACCAAGAAAGAGGATGGTGAGAGTACAG
CCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATGCCACCACCATTGTCCAGAAGACTCAGTCAACAATATTTGC
AGCACAGACGGATATTGTTTCACGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGG
ACTAGAAGGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAA
GGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTTGATGGACCTATACAC
CACAGGGCTTTACTTATATCTGTGACTGTCTGTAGTTTGCTCTTGGTCCTTATCATATTATTTTGTTACTTCCGGTA
TAAAAGACAAGAAACCAGACCTCGATACAGCATTGGGTTAGAACAGGATGAAACTTACATTCCTCCTGGAGAATCCC
TGAGAGACTTAATTGAGCAGTCTCAGAGCTCAGGAAGTGGATCAGGCCTCCCTCTGCTGGTCCAAAGGACTATAGCT
AAGCAGATTCAGATGGTGAAACAGATTGGAAAAGGTCGCTATGGGGAAGTTTGGATGGGAAAGTGGCGTGGCGAAAA
GGTAGCTGTGAAAGTGTTCTTCACCACAGAGGAAGCCAGCTGGTTCAGAGAGACAGAAATATATCAGACAGTGTTGA
TGAGGCATGAAAACATTTTGGGTTTCATTGCTGCAGATATCAAAGGGACAGGGTCCTGGACCCAGTTGTACCTAATC
ACAGACTATCATGAAAATGGTTCCCTTTATGATTATCTGAAGTCCACCACCCTAGACGCTAAATCAATGCTGAAGTT
AGCCTACTCTTCTGTCAGTGGCTTATGTCATTTACACACAGAAATCTTTAGTACTCAAGGCAAACCAGCAATTGCCC

ATCGAGATCTGAAAAGTAAAAACATTCTGGTGAAGAAAAATGGAACTTGCTGTATTGCTGACCTGGGCCTGGCTGTT
AAATTTATTAGTGATACAAATGAAGTTGACATACCACCTAACACTCGAGTTGGCACCAAACGCTATATGCCTCCAGA
AGTGTTGGACGAGAGCTTGAACAGAAATCACTTCCAGTCTTACATCATGGCTGACATGTATAGTTTTGGCCTCATCC
TTTGGGAGGTTGCTAGGAGATGTGTATCAGGAGGTATAGTGGAAGAATACCAGCTTCCTTATCATGACCTAGTGCCC
AGTGACCCCTCTTATGAGGACATGAGGGAGATTGTGTGCATCAAGAAGTTACGCCCCTCATTCCCAAACCGGTGGAG
CAGTGATGAGTGTCTAAGGCAGATGGGAAAACTCATGACAGAATGCTGGGCTCACAATCCTGCATCAAGGCTGACAG
CCCTGCGGGTTAAGAAAACACTTGCCAAAATGTCAGAGTCCCAGGACATTAAACTC(SEQ ID NO:93)

[0323] 编码加工的胞外ALK6多肽的核酸序列如下：

[0324]

AACTTCCTTGATAACATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACCAAGAAAGAGGATGGTGAGAGTAC
AGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATGCCACCACCATTGTCCAGAAGACTCAGTCAACAATATTT
GCAGCACAGACGGATATTGTTTCACGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTA
GGACTAGAAGGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGA
AAGGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTTGATGGACCTATAC
ACCACAGG(SEQ ID NO:94)

[0325] 在某些实施方案中，本公开内容涉及包含至少一种ALK6多肽的异多聚体复合体，其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的ALK6多肽(例如包含ALK6多肽的异多聚体复合体及其用途)是可溶解的(例如ALK6的胞外域)。在其它优选的实施方案中，用于本公开内容发明的ALK6多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，本公开内容的异多聚体复合体包含与SEQ ID NO:34、35、91或92的氨基酸序列有至少至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK6多肽。在一些实施方案中，本公开内容的异多聚体复合体由与SEQ ID NO:34、35、91或92的氨基酸序列有至少至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK6多肽组成或基本由其组成。

[0326] 在某些方面，本公开内容涉及包含ALK7多肽的蛋白质复合体。本文所用术语“ALK7”是指来自任何物种的激活素受体样激酶-7蛋白的家族和蛋白通过诱变或其它修饰衍生于所述ALK7的变体。本文对ALK7的提及要理解为是提及目前已鉴定形式的任一种。ALK7家族的成员一般是跨膜蛋白，由具有富含半胱氨酸的区域的配体结合胞外域、跨膜结构域和具有预测的丝氨酸/苏氨酸激酶活性的胞质结构域组成。

[0327] 术语“ALK7多肽”包括包含ALK7家族成员的任何天然存在的多肽及其保持有用活性的任何变体(包括突变体、片段、融合物和模拟肽形式)的多肽。本文所述的所有ALK7相关多肽的氨基酸的编号以下面的人ALK7前体蛋白序列(SEQ ID NO:38)的编号为基础，除非另有特别标注。

[0328] 已描述了人ALK7的4种天然存在的同种型。典型的人ALK7同种型1前体蛋白的序列(NCBIRefSeqNP_660302.2)如下：

1 MTRALCSALR QALLLLAAAA**ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI**

61 **KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PME**LAIIITV

121 PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL IYDVTASGSG

181 SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ

[0329] 241 TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA

301 SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN

361 PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD

421 MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK

481 TISQLCVKED CKA (SEQ ID NO: 38)

[0330] 信号肽用单下划线表示，胞外域用粗体字表示。

[0331] 加工的胞外ALK7同种型1多肽序列如下：

[0332]

ELSPGLKCVCLLCDSSNFTCQTEGACWASVMLTNGKEQVIKSCVSLPELNAQVFCHSSNNVTKTECCFTDFCNNITL HLPTASPNAPKLGPME(SEQ ID NO:39)

[0333] 编码人ALK7同种型1前体蛋白的核酸序列见下(SEQ ID NO:40)，相当于Genbank参比序列NM_145259.2的核苷酸244-1722。信号序列用下划线表示，胞外域用粗体字表示。

[0334]

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGAGCTCTCGCCAGGACT GAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCATGTTGGGCATCAGTCATGC TAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGT TCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATC ACCAAATGCCCCAAAACTTGGACCCATGGAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTCCTGTCCATAGCTG CGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTGCTCCTACAGGAAGAAAAAGAGACCAAATGTGGAGGAACCA CTCTCTGAGTGCAATCTGGTAAATGCTGGAAAAACTCTGAAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGG CTCTGGTCTACCTCTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGAT TTGGTGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCT TGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAA CAAAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGA ATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATG GAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTG TGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGA ATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCC TTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGT TGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTG ACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGT GAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAA AGAAGACTGCAAAGCC(SEQ ID NO:40)

[0335] 编码加工的胞外ALK7多肽(同种型1)的核酸序列如下：

[0336]

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCATG
TTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTC
AAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTG
CACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATGGAG(SEQ ID NO:41)

[0337] 呈其加工形式的人ALK7的可变同种型,同种型2的氨基酸序列(NCBI Ref Seq NP_001104501.1)如下表示(SEQ ID NO:301),其中胞外域用粗体字表示。

[0338]

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

[0339] 胞外ALK7多肽(同种型2)的氨基酸序列如下:

[0340] MLTNGKEQVIKSCVSLPELNAQVFCHSSNNVTKTECCFTDFCNNITLHLPTASPNAPKLGPME(SEQ ID NO:302)。

[0341] 编码加工的ALK7多肽(同种型2)的核酸序列见下(SEQ ID NO:303),相当于NCBI参比序列NM_001111031.1的核苷酸279-1607。胞外域用粗体字表示。

[0342]

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTC
TCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCA
ACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAA
CAACATAACACTGCACCTTCCAACAGCATCACCAAATGCCCC
AAAACTTGGACCCATGGAG

CTGGCCATCATTATTACTGTGCCTGTTTGCCTCCTGTCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCG
ACAGTGCTCCTACAGGAAGAAAAAGAGACCAAATGTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTGGAA
AAACTCTGAAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTGGTTCAAAGGACA
ATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGG
GGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGG
TCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGG
CTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGAT
CAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTA
TTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTG
GCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGC
TCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTC
TGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATG

GTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCA
GTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCC
TAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC(SEQ ID NO:303)

[0343] 编码胞外ALK7多肽(同种型2)的核酸序列如下(SEQ ID NO:304):

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCA
TAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAG
CATCACCAAATGCCCCAAAACTTGGACCCATGGAG(SEQ ID NO:304)

[0344] 可变人ALK7前体蛋白,同种型3的氨基酸序列(NCBI Ref Seq NP_001104502.1)如下表示(SEQ ID NO:305),其中信号肽用单下划线表示。

[0345]

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

[0346] 加工的ALK7多肽(同种型3)的氨基酸序列如下(SEQ ID NO:306)。该同种型缺乏跨膜结构域,因此提出作为其整体是可溶解的(Roberts等,2003,BiolReprod68:1719-1726)。如下所述预测了SEQ ID NO:306的N端变体。

[0347]

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

[0348] 编码未加工的ALK7多肽前体蛋白(同种型3)的核酸序列见下(SEQ ID NO:307),相当于NCBI参比序列NM_001111032.1的核苷酸244-1482。信号序列用实线下划线表示。

[0349]

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGAGCTCTCGCCAGGACT
GAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCATGTTGGGCATCAGTCATGC
TAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGT
TCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGGTCT
ACCTCTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGG
TGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGT
GAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAA
TGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATA
TAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTT

GGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTG
TGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAG
TGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGA
GCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTA
CCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGT
TTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGG
TATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTG
CAAAGCC(SEQ ID NO:307)

[0350] 编码加工的ALK7多肽(同种型3)的核酸序列如下(SEQ ID NO:308)：

[0351]

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCATG
TTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTC
AAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTG
CACCTTCCAACAGGTCTACCTCTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAA
AGGTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATG
AAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCT
GCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGA
CTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACC
TTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTG
AAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACAT
ACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCT
TTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGA
GGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGT
TGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAA
TAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTT
TGTGTCAAAGAAGACTGCAAAGCC(SEQ ID NO:308)

[0352] 可变人ALK7前体蛋白,同种型4的氨基酸序列(NCBI Ref Seq NP_001104503.1)如下表示(SEQ ID NO:309),其中信号肽用单下划线表示。

[0353]

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

[0354] 加工的ALK7多肽(同种型4)的氨基酸序列如下(SEQ ID NO:310)。像ALK7同种型3一样,同种型4缺乏跨膜结构域,因此提出作为其整体是可溶解的(Roberts等,2003,BiolReprod68:1719-1726)。如下所述预测了SEQ ID NO:310的N端变体。

[0355] 1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN

61 VTKTECCFTD FCNNITLHLP TDNGTWTQLW LVSEYHEQGS LYDYLNRNIV TVAGMIKLAL

121 SIASGLAHLH MEIVGTQGKP AIAHRDIKSK NILVKKCETC AIADLGLAVK HDSILNTIDI

[0356] 181 PQNPKVGTKR YMAPEMLDDT MNVNIFESFK RADIYSVGLV YWEIARRCSV GGIVEEYQLP

240 YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM GRIMRECWYA NGAARLTALR

301 IKKTISQLCV KEDCKA (SEQ ID NO: 310)

[0357] 编码未加工的ALK7多肽前体蛋白(同种型4)的核酸序列见下(SEQ ID NO:311),相当于NCBI参比序列NM_001111033.1的核苷酸244-1244。信号序列用实线下划线表示。

[0358]

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGAGCTCTCGCCAGGACT
GAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCATGTTGGGCATCAGTCATGC
TAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGT
TCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGATAA
TGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATA
TAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTT
GGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTG
TGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAG
TGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGA
GCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTA
CCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGT
TTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGG
TATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTG
CAAAGCCTAA(SEQ ID NO:311)

[0359] 编码加工的ALK7多肽(同种型4)的核酸序列如下(SEQ ID NO:312):

[0360]

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCATG
TTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTC
AAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTG
CACCTTCCAACAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGA
CTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACC
TTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTG
AAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACAT
ACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCT
TTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGA
GGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGT
TGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAA
TAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTT
TGTGTCAAAGAAGACTGCAAAGCCTAA(SEQ ID NO:312)

[0361] 根据大鼠中全长ALK7(同种型1)的信号序列(参见NCBI参比序列NP_620790.1)并根据人和大鼠ALK7之间的高度序列同一性,预测人ALK7同种型1的加工形式如下(SEQ ID

NO:313)。

[0362]
```
  1 LKCVCLLCDS SNFTCQTEGA CWASVMLTNG KEQVIKSCVS LPELNAQVFC HSSNNVTKTE
 61 CCFTDFCNNI TLHLPTASPN APKLGPME          (SEQ ID NO: 313)
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[0363] 预测其中SEQ ID NO:39在N端截短1、2、3、4、5、6或7个氨基酸和SEQ ID NO:313在N端截短1或2个氨基酸的加工的ALK7同种型1的活性变体。与SEQ ID NO:313一致，另预期亮氨酸是人ALK7同种型3(SEQ ID NO:306)和人ALK7同种型4(SEQ ID NO:310)的加工形式的N端氨基酸。

[0364] 在某些实施方案中，本公开内容涉及包含至少一种ALK7多肽的异多聚体复合体，其包括其片段、功能变体和修饰形式。优选用于本公开内容发明的ALK7多肽(例如包含ALK7多肽的异多聚体复合体及其用途)是可溶解的(例如ALK7的胞外域)。在其它优选的实施方案中，用于本公开内容发明的ALK7多肽结合和/或抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，本公开内容的异多聚体复合体包含与SEQ ID NO:38、39、112、114、301、302、305、306、309、310、313、405或406的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK7多肽。在一些实施方案中，本公开内容的异多聚体复合体由与SEQ ID NO:38、39、112、114、301、302、305、306、309、310、313、405或406的氨基酸序列有至少70％、75％、80％、85％、90％、95％、97％、98％或99％同一性的至少一种ALK7多肽组成或基本由其组成。

[0365] 在某些方面，本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK1:ActRIIB异多聚体复合体包含至少一种ALK1多肽，其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:ActRIIB异多聚体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:ActRIIB异多聚体复合体包含至少一种ActRIIB多肽，其包含与与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在某些优选的实施方案中，本公开内容的ActRIIB多肽不含酸性氨基酸(例如天然存在的D或E氨基酸或人工酸性氨基酸)。优选本公开内容的ALK1:ActRIIB异多聚体复合体是可溶解的(例如包含ALK1和/或ActRIIB的胞外域)。在其它实施方案中，本公开内容的ALK1:ActRIIB异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转

导的诱导)。在一些实施方案中,与相应的同多聚复合体(即ALK1和ActRIIB同多聚体)相比,本公开内容的ALK1:ActRIIB异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK1:ActRIIB异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK1:ActRIIB异多聚体复合体是异二聚体。

[0366] 在某些方面,本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK2:ActRIIB异多聚体复合体包含至少一种ALK2多肽,其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK2:ActRIIB异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1的氨基酸29-109有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK2:ActRIIB异多聚体复合体包含至少一种ActRIIB多肽,其包含与与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在某些优选的实施方案中,本公开内容的ActRIIB多肽不含酸性氨基酸(例如天然存在的D或E氨基酸或人工酸性氨基酸)。优选本公开内容的ALK2:ActRIIB异多聚体复合体是可溶解的(例如包含ALK2和/或ActRIIB的胞外域)。在其它实施方案中,本公开内容的ALK2:ActRIIB异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK2和ActRIIB同多聚体)相比,本公开内容的ALK2:ActRIIB异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK2:ActRIIB异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK2:ActRIIB异多聚体复合体是异二聚体。

[0367] 在某些方面,本公开内容涉及包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK3:ActRIIB异多聚体复合体包含至少一种ALK3多肽,其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK3:ActRIIB异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1的氨基酸29-109有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、

96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK3:ActRIIB异多聚体复合体包含至少一种ActRIIB多肽，其包含与与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在某些优选的实施方案中，本公开内容的ActRIIB多肽不含酸性氨基酸(例如天然存在的D或E氨基酸或人工酸性氨基酸)。优选本公开内容的ALK3:ActRIIB异多聚体复合体是可溶解的(例如包含ALK3和/或ActRIIB的胞外域)。在其它实施方案中，本公开内容的ALK3:ActRIIB异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK3和ActRIIB同多聚体)相比，本公开内容的ALK3:ActRIIB异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK3:ActRIIB异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK3:ActRIIB异多聚体复合体是异二聚体。

[0368] 在某些方面，本公开内容涉及包含至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK4:ActRIIB异多聚体复合体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK4:ActRIIB异多聚体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK4:ActRIIB异多聚体复合体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在某些优选的实施方案中，本公开内容的ActRIIB多肽不含酸性氨基酸(例如天然存在的D或E氨基酸或人工酸性氨基酸)。优选本公开内容的ALK4:ActRIIB异多聚体复合体是可溶解的(例如包含ALK4和/或ActRIIB的胞外域)。在其它实施方案中，本公开内容的ALK4:ActRIIB异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/

8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK4和ActRIIB同多聚体)相比,本公开内容的ALK4:ActRIIB异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK4:ActRIIB异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK4:ActRIIB异多聚体复合体是异二聚体。

[0369] 在某些方面,本公开内容涉及包含至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK5:ActRIIB异多聚体复合体包含至少一种ALK5多肽,其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK5:ActRIIB异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1的氨基酸29-109有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK5:ActRIIB异多聚体复合体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在某些优选的实施方案中,本公开内容的ActRIIB多肽不含酸性氨基酸(例如天然存在的D或E氨基酸或人工酸性氨基酸)。优选本公开内容的ALK5:ActRIIB异多聚体复合体是可溶解的(例如包含ALK5和/或ActRIIB的胞外域)。在其它实施方案中,本公开内容的ALK5:ActRIIB异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK5和ActRIIB同多聚体)相比,本公开内容的ALK5:ActRIIB异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK5:ActRIIB异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK5:ActRIIB异多聚体复合体是异二聚体。

[0370] 在某些方面,本公开内容涉及包含至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK6:ActRIIB异多聚体复合体包含至少一种ALK6多肽,其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK6:ActRIIB异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1的氨基酸29-109有至少70%、75%、80%、

85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK6:ActRIIB异多聚体复合体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在某些优选的实施方案中，本公开内容的ActRIIB多肽不含酸性氨基酸(例如天然存在的D或E氨基酸或人工酸性氨基酸)。优选本公开内容的ALK6:ActRIIB异多聚体复合体是可溶解的(例如包含ALK6和/或ActRIIB的胞外域)。在其它实施方案中，本公开内容的ALK6:ActRIIB异多聚体结合和/或抑制(拮抗)的一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK6和ActRIIB同多聚体)相比，本公开内容的ALK6:ActRIIB异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK6:ActRIIB异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK6:ActRIIB异多聚体复合体是异二聚体。

[0371] 在某些方面，本公开内容涉及包含至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK7:ActRIIB异多聚体复合体包含至少一种ALK7多肽，其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305和309的氨基酸28-92有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK7:ActRIIB异多聚体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1的氨基酸29-109有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK7:ActRIIB异多聚体复合体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在某些优选的实施方案中，本公开内容的ActRIIB多肽不含酸性氨基酸(例如天然存在的D或E氨基酸或人工酸性氨基酸)。优选本公开内容的ALK7:ActRIIB异多聚体复合体是可溶解的(例如包含ALK7和/或ActRIIB的胞外域)。在其它实施方案中，本公开内容的ALK7:ActRIIB异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性

(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK7和ActRIIB同多聚体)相比,本公开内容的ALK7:ActRIIB异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK7:ActRIIB异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK7:ActRIIB异多聚体复合体是异二聚体。

[0372] 在某些方面,本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK1:ActRIIA异多聚体包含至少一种ALK1多肽,其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK1:ActRIIA异多聚体包含至少一种ActRIIA多肽,其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ActRII异多聚体是可溶解的(例如包含ALK1和/或ActRIIA的胞外域)。在其它优选的实施方案中,本公开内容的ALK1:ActRIIA异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK1和ActRIIA同多聚体)相比,本公开内容的ALK1:ActRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ActRIIA异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK1:ActRIIA异多聚体复合体是异二聚体。

[0373] 在某些方面,本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK2:ActRIIA异多聚体包含至少一种ALK2多肽,其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK2:ActRIIA异多聚体包含至少一种ActRIIA多肽,其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:ActRII异多聚体是可溶解的(例如包含ALK2和/或ActRIIA的胞外域)。在其它优选的实施方案中,本公开内容的ALK2:ActRIIA异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、

BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK2和ActRIIA同多聚体)相比,本公开内容的ALK2:ActRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK2:ActRIIA异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK2:ActRIIA异多聚体复合体是异二聚体。

[0374] 在某些方面,本公开内容涉及包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK3:ActRIIA异多聚体包含至少一种ALK3多肽,其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK3:ActRIIA异多聚体包含至少一种ActRIIA多肽,其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK3:ActRII异多聚体是可溶解的(例如包含ALK3和/或ActRIIA的胞外域)。在其它优选的实施方案中,本公开内容的ALK3:ActRIIA异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK3和ActRIIA同多聚体)相比,本公开内容的ALK3:ActRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK3:ActRIIA异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK3:ActRIIA异多聚体复合体是异二聚体。

[0375] 在某些方面,本公开内容涉及包含至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK4:ActRIIA异多聚体包含至少一种ALK4多肽,其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK4:ActRIIA异多聚体包含至少一种ActRIIA多肽,其包含与成SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组。优选本公开内容的

ALK4:ActRII异多聚体是可溶解的(例如包含ALK4和/或ActRIIA的胞外域)。在其它优选的实施方案中,本公开内容的ALK4:ActRIIA异多聚体复合体结合和/或抑制(拮抗)的一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK4和ActRIIA同多聚体)相比,本公开内容的ALK4:ActRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK4:ActRIIA异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK4:ActRIIA异多聚体复合体是异二聚体。

[0376] 在某些方面,本公开内容涉及包含至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK5:ActRIIA异多聚体包含至少一种ALK5多肽,其包含SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK5:ActRIIA异多聚体包含至少一种ActRIIA多肽,其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK5:ActRII异多聚体是可溶解的(例如包含ALK5和/或ActRIIA的胞外域)。在其它优选的实施方案中,本公开内容的ALK5:ActRIIA异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK5和ActRIIA同多聚体)相比,本公开内容的ALK5:ActRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK5:ActRIIA异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK5:ActRIIA异多聚体复合体是异二聚体。

[0377] 在某些方面,本公开内容涉及包含至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK6:ActRIIA异多聚体包含至少一种ALK6多肽,其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK6:ActRIIA异多聚体包含至

少一种ActRIIA多肽，其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK6:ActRII异多聚体是可溶解的(例如包含ALK6和/或ActRIIA的胞外域)。在其它优选的实施方案中，本公开内容的ALK6:ActRIIA异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK6和ActRIIA同多聚体)相比，本公开内容的ALK6:ActRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK6:ActRIIA异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK6:ActRIIA异多聚体复合体是异二聚体。

[0378] 在某些方面，本公开内容涉及包含至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK7:ActRIIA异多聚体包含至少一种ALK7多肽，其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-93有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK7:ActRIIA异多聚体包含至少一种ActRIIA多肽，其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK7:ActRII异多聚体是可溶解的(例如包含ALK7和/或ActRIIA的胞外域)。在其它优选的实施方案中，本公开内容的ALK7:ActRIIA异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合(即ALK7和ActRIIA同多聚体)体相比，本公开内容的ALK7:ActRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK7:ActRIIA异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK7:ActRIIA异多聚体复合体是异二聚体。

[0379] 在某些方面，本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在

一些实施方案中，本公开内容的ALK1:TGFBRII异多聚体包含至少一种ALK1多肽，其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:TGFBRII异多聚体复合体包含至少一种TGFBRII多肽，其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:TGFBRII异多聚体复合体是可溶解的(例如包含ALK1和/或TGFBRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:TGFBRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合(即ALK1和TGFBRII同多聚体)体相比，本公开内容的ALK1:TGFBRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK1:TGFBRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK1:TGFBRII异多聚体复合体是异二聚体。

[0380] 在某些方面，本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK2:TGFBRII异多聚体包含至少一种ALK2多肽，其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK2:TGFBRII异多聚体复合体包含至少一种TGFBRII多肽，其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:TGFBRII异多聚体复合体是可溶解的(例如包含ALK2和/或TGFBRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK2:TGFBRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK2和TGFBRII同多聚体)相比，本公开内容的

ALK2:TGFBRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK2:TGFBRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK2:TGFBRII异多聚体复合体是异二聚体。

[0381] 在某些方面，本公开内容涉及包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK3:TGFBRII异多聚体包含至少一种ALK3多肽，其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK3:TGFBRII异多聚体复合体包含至少一种TGFBRII多肽，其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK3:TGFBRII异多聚体复合体是可溶解的(例如包含ALK3和/或TGFBRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK3:TGFBRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK3和TGFBRII同多聚体)相比，本公开内容的ALK3:TGFBRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK3:TGFBRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK3:TGFBRII异多聚体复合体是异二聚体。

[0382] 在某些方面，本公开内容涉及包含至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK4:TGFBRII异多聚体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK4:TGFBRII异多聚体复合体包含至少一种TGFBRII多肽，其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK4:TGFBRII异多聚体复合体是可溶解的(例如包含ALK4和/或TGFBRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK4:TGFBRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、

BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK4和TGFBRII同多聚体)相比,本公开内容的ALK4:TGFBRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK4:TGFBRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK4:TGFBRII异多聚体复合体是异二聚体。

[0383] 在某些方面,本公开内容涉及包含至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK5:TGFBRII异多聚体包含至少一种ALK5多肽,其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK5:TGFBRII异多聚体复合体包含至少一种TGFBRII多肽,其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK5:TGFBRII异多聚体复合体是可溶解的(例如包含ALK5和/或TGFBRII的胞外域)。在其它优选的实施方案中,本公开内容的ALK5:TGFBRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK5和TGFBRII同多聚体)相比,本公开内容的ALK5:TGFBRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK5:TGFBRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK5:TGFBRII异多聚体复合体是异二聚体。

[0384] 在某些方面,本公开内容涉及包含至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK6:TGFBRII异多聚体包含至少一种ALK6多肽,其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK6:TGFBRII异多聚体复合体

包含至少一种TGFBRII多肽，其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK6:TGFBRII异多聚体复合体是可溶解的(例如包含ALK6和/或TGFBRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK6:TGFBRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK6和TGFBRII同多聚体)相比，本公开内容的ALK6:TGFBRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK6:TGFBRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK6:TGFBRII异多聚体复合体是异二聚体。

[0385] 在某些方面，本公开内容涉及包含至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK7:TGFBRII异多聚体包含至少一种ALK7多肽，其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-93有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK7:TGFBRII异多聚体复合体包含至少一种TGFBRII多肽，其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK7:TGFBRII异多聚体复合体是可溶解的(例如包含ALK7和/或TGFBRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK7:TGFBRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK7和TGFBRII同多聚体)相比，本公开内容的ALK7:TGFBRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK7:TGFBRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK7:TGFBRII异多聚体复合体是异二聚体。

[0386] 在某些方面，本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK1:MISRII异多聚体包含至少一种ALK1多肽，其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:MISRII异多聚体复合体包含至少一种MISRII多肽，其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:MISRII异多聚体复合体是可溶解的(例如包含ALK1和/或MISRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:MISRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK1和MISRII同多聚体)相比，本公开内容的ALK1:MISRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK1:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK1:MISRII异多聚体是异二聚体。

[0387] 在某些方面，本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK2:MISRII异多聚体包含至少一种ALK2多肽，其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK2:MISRII异多聚体复合体包含至少一种MISRII多肽，其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:MISRII异多聚体复合体是可溶解的(例如包含ALK2和/或MISRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK2:MISRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK2和MISRII同多聚

体)相比,本公开内容的ALK2:MISRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK2:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK2:MISRII异多聚体是异二聚体。

[0388] 在某些方面,本公开内容涉及异多聚体包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)。在一些实施方案中,本公开内容的ALK3:MISRII异多聚体包含至少一种ALK3多肽,其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK3:MISRII异多聚体复合体包含至少一种MISRII多肽,其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK3:MISRII异多聚体复合体是可溶解的(例如包含ALK3和/或MISRII的胞外域)。在其它优选的实施方案中,本公开内容的ALK3:MISRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK3和MISRII同多聚体)相比,本公开内容的ALK3:MISRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK3:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK3:MISRII异多聚体是异二聚体。

[0389] 在某些方面,本公开内容涉及包含至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK4:MISRII异多聚体包含至少一种ALK4多肽,其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK4:MISRII异多聚体复合体包含至少一种MISRII多肽,其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK4:MISRII异多聚体复合体是可溶解的(例如包含ALK4和/或MISRII的胞外域)。在其它优选的实施方案中,本公开内容的ALK4:MISRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激

活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)。在一些实施方案中，本公开内容的ALK4:MISRII异多聚体复合体具有不同的配体结合特异性/特征与其相应的同多聚复合体相比，(即ALK4和MISRII同多聚体)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。本公开内容的ALK4:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK4:MISRII异多聚体是异二聚体。

[0390] 在某些方面，本公开内容涉及包含至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK5:MISRII异多聚体包含至少一种ALK5多肽，其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK5:MISRII异多聚体复合体包含至少一种MISRII多肽，其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK5:MISRII异多聚体复合体是可溶解的(例如包含ALK5和/或MISRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK5:MISRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK5和MISRII同多聚体)相比，本公开内容的ALK5:MISRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK5:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK5:MISRII异多聚体是异二聚体。

[0391] 在某些方面，本公开内容涉及包含至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK6:MISRII异多聚体包含至少一种ALK6多肽，其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK6:MISRII异多聚体复合体包含至少一种MISRII多肽，其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性

的序列、由其组成或基本由其组成。优选本公开内容的ALK6:MISRII异多聚体复合体是可溶解的(例如包含ALK6和/或MISRII的胞外域)。在其它优选的实施方案中,本公开内容的ALK6:MISRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK6和MISRII同多聚体)相比,本公开内容的ALK6:MISRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK6:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK6:MISRII异多聚体是异二聚体。

[0392] 在某些方面,本公开内容涉及包含至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK7:MISRII异多聚体包含至少一种ALK7多肽,其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-93有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK7:MISRII异多聚体复合体包含至少一种MISRII多肽,其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK7:MISRII异多聚体复合体是可溶解的(例如包含ALK7和/或MISRII的胞外域)。在其它优选的实施方案中,本公开内容的ALK7:MISRII异多聚体复合体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK7和MISRII同多聚体)相比,本公开内容的ALK7:MISRII异多聚体复合体具有不同的配体结合特异性/特征。本公开内容的ALK7:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK7:MISRII异多聚体是异二聚体。

[0393] 在某些方面,本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK1:BMPRII异多聚体复合体包含至少一种ALK1多肽,其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、

96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:BMPRII异多聚体复合体包含至少一种BMPRII多肽，其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:BMPRII异多聚体是可溶解的(例如包含ALK1和/或BMPRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:BMPRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK1和BMPRII同多聚体)相比，本公开内容的ALK1:BMPRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:BMPRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK1:BMPRII异多聚体复合体是异二聚体。

[0394] 在某些方面，本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK2:BMPRII异多聚体复合体包含至少一种ALK2多肽，其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK2:BMPRII异多聚体复合体包含至少一种BMPRII多肽，其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:BMPRII异多聚体是可溶解的(例如包含ALK2和/或BMPRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK2:BMPRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK2和BMPRII同多聚体)相比，本公开内容的ALK2:BMPRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK2:BMPRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK2:BMPRII异多聚体复合体是异二聚体。

[0395] 在某些方面，本公开内容涉及包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一

些实施方案中，本公开内容的ALK3:BMPRII异多聚体复合体包含至少一种ALK3多肽，其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK3:BMPRII异多聚体复合体包含至少一种BMPRII多肽，其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK3:BMPRII异多聚体是可溶解的(例如包含ALK3和/或BMPRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK3:BMPRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK3和BMPRII同多聚体)相比，本公开内容的ALK3:BMPRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK3:BMPRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK3:BMPRII异多聚体复合体是异二聚体。

[0396] 在某些方面，本公开内容涉及包含至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK4:BMPRII异多聚体复合体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK4:BMPRII异多聚体复合体包含至少一种BMPRII多肽，其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK4:BMPRII异多聚体是可溶解的(例如包含ALK4和/或BMPRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK4:BMPRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK4和BMPRII同多聚体)相比，本公开内容的ALK4:BMPRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK4:BMPRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体

结构。在某些优选的实施方案中，本公开内容的ALK4:BMPRII异多聚体复合体是异二聚体。

[0397] 在某些方面，本公开内容涉及包含至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK5:BMPRII异多聚体复合体包含至少一种ALK5多肽，其包含与SEQ ID NO:SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK5:BMPRII异多聚体复合体包含至少一种BMPRII多肽，其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK5:BMPRII异多聚体是可溶解的(例如包含ALK5和/或BMPRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK5:BMPRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK5和BMPRII同多聚体)相比，本公开内容的ALK5:BMPRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK5:BMPRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK5:BMPRII异多聚体复合体是异二聚体。

[0398] 在某些方面，本公开内容涉及包含至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK6:BMPRII异多聚体复合体包含至少一种ALK6多肽，其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK6:BMPRII异多聚体复合体包含至少一种BMPRII多肽，其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK6:BMPRII异多聚体是可溶解的(例如包含ALK6和/或BMPRII的胞外域)。在其它优选的实施方案中，本公开内容的ALK6:BMPRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍

生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK6和BMPRII同多聚体)相比,本公开内容的ALK6:BMPRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK6:BMPRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK6:BMPRII异多聚体复合体是异二聚体。

[0399] 在某些方面,本公开内容涉及包含至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK7:BMPRII异多聚体复合体包含至少一种ALK7多肽,其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-92有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK7:BMPRII异多聚体复合体包含至少一种BMPRII多肽,其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK7:BMPRII异多聚体是可溶解的(例如包含ALK7和/或BMPRII的胞外域)。在其它优选的实施方案中,本公开内容的ALK6:BMPRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK7和BMPRII同多聚体)相比,本公开内容的ALK7:BMPRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK7:BMPRII异多聚体复合体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK7:BMPRII异多聚体复合体是异二聚体。

[0400] 在某些方面,本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK1:ALK2异多聚体包含至少一种ALK1多肽,其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK1:ALK2异多聚体包含至少一种ALK2多肽,其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ALK2异多聚体是可溶解的(例如包含ALK1和/或ALK2的胞外域)。在其它优选的实施方案中,本公开内容的ALK1:

ALK2异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK1和ALK2同多聚体)相比,本公开内容的ALK1:ALK2异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ALK2异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK1:ALK2异多聚体是异二聚体。

[0401] 在某些方面,本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK1:ALK2异多聚体包含至少一种ALK1多肽,其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK1:ALK2异多聚体包含至少一种ALK2多肽,其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ALK2异多聚体是可溶解的(例如包含ALK1和/或ALK2的胞外域)。在其它优选的实施方案中,本公开内容的ALK1:ALK2异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK1和ALK2同多聚体)相比,本公开内容的ALK1:ALK2异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ALK2异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK1:ALK2异多聚体是异二聚体。

[0402] 在某些方面,本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK1:ALK3异多聚体包含至少一种ALK1多肽,其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK1:ALK3异多聚体包含至少一种ALK3多肽,其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至

少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ALK3异多聚体是可溶解的(例如包含ALK1和/或ALK3的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:ALK3异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK1和ALK3同多聚体)相比，本公开内容的ALK1:ALK3异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ALK3异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK1:ALK3异多聚体是异二聚体。

[0403] 在某些方面，本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK1:ALK4异多聚体包含至少一种ALK1多肽，其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:ALK4异多聚体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ALK4异多聚体是可溶解的(例如包含ALK1和/或ALK4的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:ALK4异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK1和ALK4同多聚体)相比，本公开内容的ALK1:ALK4异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ALK4异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK1:ALK4异多聚体是异二聚体。

[0404] 在某些方面，本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK1:ALK4异多聚体包含至少一种ALK1多肽，其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、

98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:ALK4异多聚体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ALK4异多聚体是可溶解的(例如包含ALK1和/或ALK4的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:ALK4异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK1和ALK4同多聚体)相比，本公开内容的ALK1:ALK4异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ALK4异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK1:ALK4异多聚体是异二聚体。

[0405] 在某些方面，本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK1:ALK5异多聚体包含至少一种ALK1多肽，其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:ALK5异多聚体包含至少一种ALK5多肽，其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ALK5异多聚体是可溶解的(例如包含ALK1和/或ALK5的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:ALK5异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK1和ALK5同多聚体)相比，本公开内容的ALK1:ALK5异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ALK5异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK1:ALK5异多聚体是异二聚体。

[0406] 在某些方面，本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些

实施方案中，本公开内容的ALK1:ALK6异多聚体包含至少一种ALK1多肽，其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:ALK6异多聚体包含至少一种ALK6多肽，其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ALK6异多聚体是可溶解的(例如包含ALK1和/或ALK6的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:ALK6异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK1和ALK6同多聚体)相比，本公开内容的ALK1:ALK6异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ALK6异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK1:ALK6异多聚体是异二聚体。

[0407] 在某些方面，本公开内容涉及包含至少一种ALK1多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK1:ALK7异多聚体包含至少一种ALK1多肽，其包含与SEQ ID NO:14、15、124、126、171、172、413、414、463和464中任一个的氨基酸序列或SEQ ID NO:14的氨基酸34-95有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK1:ALK7异多聚体包含至少一种ALK7多肽，其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-92有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK1:ALK7异多聚体是可溶解的(例如包含ALK1和/或ALK7的胞外域)。在其它优选的实施方案中，本公开内容的ALK1:ALK7异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK1和ALK7同多聚体)相比，本公开内容的ALK1:ALK7异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK1:ALK7异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案

中，本公开内容的ALK1:ALK7异多聚体是异二聚体。

[0408] 在某些方面，本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK2:ALK3异多聚体包含至少一种ALK2多肽，其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK2:ALK3异多聚体包含至少一种ALK3多肽，其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:ALK3异多聚体是可溶解的(例如包含ALK2和/或ALK3的胞外域)。在其它优选的实施方案中，本公开内容的ALK2:ALK3异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK2和ALK3同多聚体)相比，本公开内容的ALK2:ALK3异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK2:ALK3异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK2:ALK3异多聚体是异二聚体。

[0409] 在某些方面，本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK4多肽(这包括其片段、功能变体和修饰形式的异多聚体)。在一些实施方案中，本公开内容的ALK2:ALK4异多聚体包含至少一种ALK2多肽，其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK2:ALK4异多聚体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:ALK4异多聚体是可溶解的(例如包含ALK2和/或ALK4的胞外域)。在其它优选的实施方案中，本公开内容的ALK2:ALK4异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相

应的同多聚复合体(即ALK2和ALK4同多聚体)相比，本公开内容的ALK2:ALK4异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK2:ALK4异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK2:ALK4异多聚体是异二聚体。

[0410] 在某些方面，本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK2:ALK5异多聚体包含至少一种ALK2多肽，其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK2:ALK5异多聚体包含至少一种ALK5多肽，其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:ALK5异多聚体是可溶解的(例如包含ALK2和/或ALK5的胞外域)。在其它优选的实施方案中，本公开内容的ALK2:ALK5异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK2和ALK5同多聚体)相比，本公开内容的ALK2:ALK5异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK2:ALK5异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK2:ALK5异多聚体是异二聚体。

[0411] 在某些方面，本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK2:ALK6异多聚体包含至少一种ALK2多肽，其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK2:ALK6异多聚体包含至少一种ALK6多肽，其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:ALK6异多聚体是可溶解的(例如包含ALK2和/或ALK6的胞外域)。在其它优选的实施方案中，本公开内容的ALK2:ALK6异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、

TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK2和ALK6同多聚体)相比,本公开内容的ALK2:ALK6异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK2:ALK6异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK2:ALK6异多聚体是异二聚体。

[0412] 在某些方面,本公开内容涉及包含至少一种ALK2多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK2:ALK7异多聚体包含至少一种ALK2多肽,其包含与SEQ ID NO:18、19、136、138、173、174、421、422、465和466中任一个的氨基酸序列或SEQ ID NO:18的氨基酸35-99有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK2:ALK7异多聚体包含至少一种ALK7多肽,其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-92有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK2:ALK7异多聚体是可溶解的(例如包含ALK2和/或ALK7的胞外域)。在其它优选的实施方案中,本公开内容的ALK2:ALK7异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK2和ALK7同多聚体)相比,本公开内容的ALK2:ALK7异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK2:ALK7异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK2:ALK7异多聚体是异二聚体。

[0413] 在某些方面,本公开内容涉及包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK3:ALK4异多聚体包含至少一种ALK3多肽,其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK3:ALK4异多聚体包含至少一种ALK4多肽,其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK3:ALK4异多聚体是可溶解的(例如包含ALK3和/或ALK4的胞外域)。在其它优选的实施方案中,本公

开内容的ALK3:ALK4异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK3和ALK4同多聚体)相比,本公开内容的ALK3:ALK4异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK3:ALK4异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK3:ALK4异多聚体是异二聚体。

[0414] 在某些方面,本公开内容涉及包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK3:ALK5异多聚体包含至少一种ALK3多肽,其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK3:ALK5异多聚体包含至少一种ALK5多肽,其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK3:ALK5异多聚体是可溶解的(例如包含ALK3和/或ALK5的胞外域)。在其它优选的实施方案中,本公开内容的ALK3:ALK5异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK3和ALK5同多聚体)相比,本公开内容的ALK3:ALK5异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK3:ALK5异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK3:ALK5异多聚体是异二聚体。

[0415] 在某些方面,本公开内容涉及包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ALK3:ALK6异多聚体包含至少一种ALK3多肽,其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ALK3:ALK6异多聚体包含至少一种ALK6多肽,其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474或SEQ ID NO:34的氨基酸32-102中任一个的氨基

酸序列或SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK3:ALK6异多聚体是可溶解的(例如包含ALK3和/或ALK6的胞外域)。在其它优选的实施方案中，本公开内容的ALK3:ALK6异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK3和ALK6同多聚体)相比，本公开内容的ALK3:ALK6异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK3:ALK6异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK3:ALK6异多聚体是异二聚体。

[0416] 在某些方面，本公开内容涉及包含至少一种ALK3多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK3:ALK7异多聚体包含至少一种ALK3多肽，其包含与SEQ ID NO:22、23、115、117、175、176、407、408、467和468中任一个的氨基酸序列或SEQ ID NO:22的氨基酸61-130有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK3:ALK7异多聚体包含至少一种ALK7多肽，其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-92有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK3:ALK7异多聚体是可溶解的(例如包含ALK3和/或ALK7的胞外域)。在其它优选的实施方案中，本公开内容的ALK3:ALK7异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK3和ALK7同多聚体)相比，本公开内容的ALK3:ALK7异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK3:ALK7异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK3:ALK7异多聚体是异二聚体。

[0417] 在某些方面，本公开内容涉及包含至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK4:ALK5异多聚体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、

95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK4:ALK5异多聚体包含至少一种ALK5多肽，其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK4:ALK5异多聚体是可溶解的(例如包含ALK4和/或ALK5的胞外域)。在其它优选的实施方案中，本公开内容的ALK4:ALK5异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK4和ALK5同多聚体)相比，本公开内容的ALK4:ALK5异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK4:ALK5异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK4:ALK5异多聚体是异二聚体。

[0418] 在某些方面，本公开内容涉及包含至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK4:ALK6异多聚体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK4:ALK6异多聚体包含至少一种ALK6多肽，其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK4:ALK6异多聚体是可溶解的(例如包含ALK4和/或ALK6的胞外域)。在其它优选的实施方案中，本公开内容的ALK4:ALK6异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)本公开内容的ALK4:ALK6异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK4:ALK6异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK4:ALK6异多聚体是异二聚体。

[0419] 在某些方面，本公开内容涉及包含至少一种ALK4多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些

实施方案中，本公开内容的ALK4:ALK7异多聚体包含至少一种ALK4多肽，其包含与SEQ ID NO:26、27、83、84、104、106、177、178、403、404、469和470中任一个的氨基酸序列或SEQ ID NO:26或83的氨基酸34-101有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK4:ALK7异多聚体包含至少一种ALK7多肽，其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-92有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK4:ALK7异多聚体是可溶解的(例如包含ALK4和/或ALK7的胞外域)。在其它优选的实施方案中，本公开内容的ALK4:ALK7异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK4和ALK7同多聚体)相比，本公开内容的ALK4:ALK7异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK4:ALK7异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK4:ALK7异多聚体是异二聚体。

[0420] 在某些方面，本公开内容涉及包含至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK5:ALK6异多聚体包含至少一种ALK5多肽，其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK5:ALK6异多聚体包含至少一种ALK6多肽，其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK5:ALK6异多聚体是可溶解的(例如包含ALK5和/或ALK6的胞外域)。在其它优选的实施方案中，本公开内容的ALK5:ALK6异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK5和ALK6同多聚体)相比，本公开内容的ALK5:ALK6异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK5:ALK6异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某

些优选的实施方案中，本公开内容的ALK5:ALK6异多聚体是异二聚体。

[0421] 在某些方面，本公开内容涉及包含至少一种ALK5多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK5:ALK7异多聚体包含至少一种ALK5多肽，其包含与SEQ ID NO:30、31、87、88、139、141、179、180、423、424、471和472中任一个的氨基酸序列或SEQ ID NO:30或87的氨基酸36-106有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK5:ALK7异多聚体包含至少一种ALK7多肽，其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-92有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK5:ALK7异多聚体是可溶解的(例如包含ALK5和/或ALK7的胞外域)。在其它优选的实施方案中，本公开内容的ALK5:ALK7异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ALK5和ALK7同多聚体)相比，本公开内容的ALK5:ALK7异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK5:ALK7异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ALK5:ALK7异多聚体是异二聚体。

[0422] 在某些方面，本公开内容涉及包含至少一种ALK6多肽(这包括其片段、功能变体和修饰形式)和至少一种ALK7多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ALK6:ALK7异多聚体包含至少一种ALK6多肽，其包含与SEQ ID NO:34、35、91、92、142、144、181、182、425、426、473和474中任一个的氨基酸序列或SEQ ID NO:34的氨基酸32-102或SEQ ID NO:91的氨基酸62-132有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ALK6:ALK7异多聚体包含至少一种ALK7多肽，其包含与SEQ ID NO:38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475和476中任一个的氨基酸序列或SEQ ID NO:38、305或309的氨基酸28-92有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ALK5:ALK7异多聚体是可溶解的(例如包含ALK6和/或ALK7的胞外域)。在其它优选的实施方案中，本公开内容的ALK6:ALK7异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性

(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ALK6和ALK7同多聚体)相比,本公开内容的ALK6:ALK7异多聚体具有不同的配体结合特异性/特征。本公开内容的ALK6:ALK7异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ALK6:ALK7异多聚体是异二聚体。

[0423] 在某些方面,本公开内容涉及包含至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)和至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ActRIIA:ActRIIB异多聚体包含至少一种ActRIIA多肽,其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ActRIIA:ActRIIB异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列或SEQ ID NO:1的氨基酸29-109或SEQ ID NO:1的25-131有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ActRIIA:ActRIIB异多聚体是可溶解的(例如包含ActRIIA和/或ActRIIB的胞外域)。在其它优选的实施方案中,本公开内容的ActRIIA:ActRIIB异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ActRIIA和ActRIIB同多聚体)相比,本公开内容的ActRIIA:ActRIIB异多聚体具有不同的配体结合特异性/特征。本公开内容的ActRIIA:ActRIIB异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ActRIIA:ActRIIB异多聚体是异二聚体。

[0424] 在某些方面,本公开内容涉及包含至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ActRIIA:BMPRII异多聚体包含至少一种ActRIIA多肽,其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ActRIIA:BMPRII异多聚体包含至少一种BMPRII多肽,其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46和71的氨基酸34-123有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ActRIIA:BMPRII异多聚体是可溶解的(例如包含ActRIIA和/或BMPRII的胞外域)。在其它优选的实施方案中,本公开内容的ActRIIA:BMPRII异多聚体结合和/或抑

制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,本公开内容的ActRIIA:BMPRII异多聚体具有不同的配体结合特异性/特征。与其相应的同多聚复合体(即ActRIIA和BMPRII同多聚体)相比,本公开内容的ActRIIA:BMPRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ActRIIA:BMPRII异多聚体是异二聚体。

[0425] 在某些方面,本公开内容涉及包含至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ActRIIA:TGFBRII异多聚体包含至少一种ActRIIA多肽,其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ActRIIA:TGFBRII异多聚体包含至少一种TGFBRII多肽,其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的ActRIIA:TGFBRII异多聚体是可溶解的(例如包含ActRIIA和/或BMPRII的胞外域)。在其它优选的实施方案中,本公开内容的ActRIIA:TGFBRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即ActRIIA和TGFBRII同多聚体)相比,本公开内容的ActRIIA:TGFBRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ActRIIA:TGFBRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ActRIIA:TGFBRII异多聚体是异二聚体。

[0426] 在某些方面,本公开内容涉及包含至少一种ActRIIA多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的ActRIIA:MISRII异多聚体包含至少一种ActRIIA多肽,其包含与SEQ ID NO:9、10、11、118、120、151、152、409、410、451和452中任一个的氨基酸序列或SEQ ID NO:9的氨基酸30-110有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的ActRIIA:MISRII异多聚体包含至少一种MISRII多肽,其包含

与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75和79的氨基酸24-116有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ActRIIA:MISRII异多聚体是可溶解的(例如包含ActRIIA和/或MISRII的胞外域)。在其它优选的实施方案中，本公开内容的ActRIIA:MISRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ActRIIA和MISRII同多聚体)相比，本公开内容的ActRIIA:MISRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ActRIIA:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ActRIIA:MISRII异多聚体是异二聚体。

[0427] 在某些方面，本公开内容涉及包含至少一种ActRIIB多肽(这包括其片段、功能变体和修饰形式)和至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ActRIIB:BMPRII异多聚体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列或SEQ ID NO:1的氨基酸29-109或SEQ ID NO:1的25-131有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ActRIIB:BMPRII异多聚体包含至少一种BMPRII多肽，其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46和71的氨基酸34-123有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ActRIIB:BMPRII异多聚体是可溶解的(例如包含ActRIIB和/或BMPRII的胞外域)。在其它优选的实施方案中，本公开内容的ActRIIB:BMPRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ActRIIB和BMPRII同多聚体)相比，本公开内容的ActRIIB:BMPRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ActRIIB:BMPRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ActRIIB:BMPRII异多聚体是异二聚体。

[0428] 在某些方面，本公开内容涉及包含至少一种ActRIIB多肽(其包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚

体。在一些实施方案中，本公开内容的ActRIIB:TGFBRII异多聚体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列或SEQ ID NO:1的氨基酸29-109或SEQ ID NO:1的25-131有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ActRIIB:TGFBRII异多聚体包含至少一种TGFBRII多肽，其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ActRIIB:TGFBRII异多聚体是可溶解的(例如包含ActRIIB和/或TGFBRII的胞外域)。在其它优选的实施方案中，本公开内容的ActRIIB:TGFBRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相应的同多聚复合体(即ActRIIB和TGFBRII同多聚体)相比，本公开内容的ActRIIB:TGFBRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ActRIIB:TGFBRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中，本公开内容的ActRIIB:TGFBRII异多聚体是异二聚体。

[0429] 在某些方面，本公开内容涉及包含至少一种ActRIIB多肽(其包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中，本公开内容的ActRIIB:MISRII异多聚体包含至少一种ActRIIB多肽，其包含与SEQ ID NO:1、2、3、4、5、6、100、102、153、154、401、402、453和454中任一个的氨基酸序列或SEQ ID NO:1的氨基酸29-109或SEQ ID NO:1的25-131有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。在一些实施方案中，本公开内容的ActRIIB:MISRII异多聚体包含至少一种MISRII多肽，其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75和79的氨基酸24-116有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的序列、由其组成或基本由其组成。优选本公开内容的ActRIIB:MISRII异多聚体是可溶解的(例如包含ActRIIB和/或MISRII的胞外域)。在其它优选的实施方案中，本公开内容的ActRIIB:MISRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中，与其相

应的同多聚复合体(即ActRIIB和MISRII同多聚体)相比,本公开内容的ActRIIB:MISRII异多聚体具有不同的配体结合特异性/特征。本公开内容的ActRIIB:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的ActRIIB:MISRII异多聚体是异二聚体。

[0430] 在某些方面,本公开内容涉及包含至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)和至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的BMPRII:TGFBRII异多聚体包含至少一种BMPRII多肽,其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的BMPRII:TGFBRII异多聚体包含至少一种TGFBRII多肽,其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的BMPRII:TGFBRII异多聚体是可溶解的(例如包含BMPRII和/或TGFBRII的胞外域)。在其它优选的实施方案中,本公开内容的BMPRII:TGFBRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即BMPRII和TGFBRII同多聚体)相比,本公开内容的BMPRII:TGFBRII异多聚体具有不同的配体结合特异性/特征。本公开内容的BMPRII:TGFBRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的BMPRII:TGFBRII异多聚体是异二聚体。

[0431] 在某些方面,本公开内容涉及包含至少一种BMPRII多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的BMPRII:MISRII异多聚体包含至少一种BMPRII多肽,其包含与SEQ ID NO:46、47、71、72、121、123、155、156、411、412、455和456中任一个的氨基酸序列或SEQ ID NO:46或71的氨基酸34-123有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的BMPRII:MISRII异多聚体包含至少一种MISRII多肽,其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的BMPRII:MISRII异多聚体是可溶解的(例如包含BMPRII和/或MISRII的胞外域)。在其它优选的实施方案中,本公开内容的BMPRII:MISRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、

BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即BMPRII和MISRII同多聚体)相比,本公开内容的BMPRII:MISRII异多聚体具有不同的配体结合特异性/特征。本公开内容的BMPRII:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的BMPRII:MISRII异多聚体是异二聚体。

[0432] 在某些方面,本公开内容涉及包含至少一种TGFBRII多肽(这包括其片段、功能变体和修饰形式)和至少一种MISRII多肽(这包括其片段、功能变体和修饰形式)的异多聚体。在一些实施方案中,本公开内容的TGFBRII:MISRII异多聚体包含至少一种TGFBRII多肽,其包含与SEQ ID NO:42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的氨基酸序列或SEQ ID NO:67的氨基酸44-168或SEQ ID NO:42的氨基酸51-143有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。在一些实施方案中,本公开内容的TGFBRII:MISRII异多聚体包含至少一种MISRII多肽,其包含与SEQ ID NO:50、51、75、76、79、80、133、135、161、162、419、420、457和458中任一个的氨基酸序列或SEQ ID NO:50、75或79的氨基酸24-116有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的序列、由其组成或基本由其组成。优选本公开内容的TGFBRII:MISRII异多聚体是可溶解的(例如包含TGFBII和/或MISRII的胞外域)。在其它优选的实施方案中,本公开内容的TGFBRII:MISRII异多聚体结合和/或抑制(拮抗)一种或多种TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的活性(例如Smad2/3和/或Smad1/5/8信号转导的诱导)。在一些实施方案中,与其相应的同多聚复合体(即TGFBRII和MISRII同多聚体)相比,本公开内容的TGFBRII:MISRII异多聚体具有不同的配体结合特异性/特征。本公开内容的TGFBRII:MISRII异多聚体包括例如异二聚体、异三聚体、异四聚体和其它的寡聚体结构。在某些优选的实施方案中,本公开内容的TGFBRII:MISRII异多聚体是异二聚体。

[0433] 在一些实施方案中,本公开内容考虑通过修饰TGF-β超家族I型受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7)和/或TGF-β超家族II型受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII)的结构制备功能变体用于诸如提高治疗效能或稳定性(例如存放期和体内对蛋白水解性降解的抗性)的目的。变体可通过氨基酸取代、缺失、添加或其组合产生。例如,有理由预期,亮氨酸用异亮氨酸或缬氨酸、天冬氨酸用谷氨酸、苏氨酸用丝氨酸的单独置换或氨基酸用结构相关氨基酸的类似置换(例如保守突变)将不会对所得分子的生物活性有重大影响。保守置换是发生在其侧链相关的氨基酸家族内的那些。可

通过评价变体多肽以类似野生型多肽的方式在细胞中产生反应或与包括例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty等一种或多种TGF-β超家族配体结合的能力,容易地确定本公开内容的多肽氨基酸序列的变化是否产生功能同系物。

[0434] 在一些实施方案中,本公开内容考虑通过修饰TGF-β超家族I型受体多肽和/或TGF-β超家族II型受体多肽的结构制备功能变体用于例如提高治疗效能或稳定性(例如延长存放期和/或增加对蛋白水解性降解的抗性)等目的。

[0435] 在某些实施方案中,本公开内容考虑本公开内容的TGF-β超家族I型受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7)和/或TGF-β超家族II型受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII)受体的特异性突变以改变多肽的糖基化。可选择所述突变以引入或消除一个或多个糖基化位点,例如O-联或N-联糖基化位点。天冬酰胺-联糖基化识别位点一般包含被合适的细胞糖基化酶特异性识别的三肽序列,天冬酰胺-X-苏氨酸或天冬酰胺-X-丝氨酸(其中"X"为任何氨基酸)。还可以通过添加一个或多个丝氨酸或苏氨酸残基至多肽序列或用一个或多个丝氨酸或苏氨酸残基取代(用于O-联糖基化位点)实现变化。在糖基化识别位点的第一或第三氨基酸位置处一个或两个的各种氨基酸取代或缺失(和/或在第二位置处的氨基酸缺失)导致修饰的三肽序列的非糖基化。增加多肽上的糖部分数目的另一种方式是通过糖苷与多肽的化学或酶促偶联。根据所采用的偶联方式,糖可连接至:(a)精氨酸和组氨酸;(b)游离羧基;(c)游离巯基,例如半胱氨酸的游离巯基;(d)游离羟基,例如丝氨酸、苏氨酸或羟脯氨酸的游离羟基;(e)芳族残基,例如苯丙氨酸、酪氨酸或色氨酸的芳族残基;或(f)谷氨酰胺的酰胺基团。脱去存在于多肽上的一个或多个糖部分可通过化学方法和/或酶促方法实现。化学去糖基化可包括例如将多肽暴露于化合物三氟甲磺酸或等同化合物中。这种处理导致大多数或全部糖的切割,只是连接糖(N-乙酰氨基葡萄糖或N-乙酰半乳糖胺)除外,同时留下完整的氨基酸序列。多肽上糖部分的酶促切割可采用Thotakura等人描述的多种内切糖苷酶和外切糖苷酶实现[Meth.Enzymol.(1987)138:350]。适当时,可调节多肽的序列,这取决于所用表达系统的类型,像哺乳动物一样,酵母、昆虫和植物细胞全都可引入可被肽的氨基酸序列影响的不同的糖基化模式。一般而言,用于人的本公开内容的TGF-β超家族I型和II受体复合体可在提供合适糖基化的哺乳动物细胞系中表达,例如HEK293或CHO细胞系,尽管预期其它哺乳动物表达细胞系也是有用的。

[0436] 本公开内容另考虑产生突变体、特别是本公开内容的TGF-β超家族I型受体多肽(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6和ALK7)和/或TGF-β超家族II型受体多肽(例如ActRIIA、ActRIIB、TGFBRII、BMPRII和MISRII)的组合突变体组以及截短突变体的方法。组合突变体库尤其可用于鉴定功能活性的(例如配体结合)TGF-β超家族I型和/或TGF-β超家族II型受体序列。筛选所述组合文库的目的可以是产生例如性质改变(例如药代动力学改变或配体结合改变)的多肽变体。下面提供多种筛选测定法和例如可用于评价变体的测定法。例如,可针对与TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、

BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)结合、防止TGF-β超家族配体与TGF-β超家族受体结合和/或干扰由TGF-β超家族配体引起的信号转导的能力来筛选TGF-β超家族I型和II受体复合体变体。

[0437] 还可在例如基于细胞的测定法或体内测定法中测试本公开内容的TGF-β超家族异多聚体复合体的活性。例如,可评价异多聚体复合体对在肌肉细胞中参与肌肉产生的基因表达或蛋白质活性。需要时,这可在一种或多种重组TGF-β超家族配体蛋白(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)存在下进行,并且可转染细胞以产生TGF-β超家族I型和II受体复合体,任选TGF-β超家族配体。同样,可将本公开内容的异多聚体复合体给予小鼠或其它动物,并可采用本领域公认的方法评价一个或多个量度,例如肌肉形成和强度。同样,可通过例如本文所述测定法和本领域已知通用知识的测定法,在成骨细胞、脂肪细胞和/或神经元细胞中针对对这些细胞生长的任何作用测试异多聚体或其变体的活性。SMAD反应性报道基因可用于所述细胞系以监测对下游信号转导的作用。

[0438] 可产生相对于参比TGF-β超家族异多聚体复合体选择性提高或效能总体提高的组合衍生变体。这类变体,当从重组DNA构建体表达时,可用于基因疗法方案。同样,诱变可产生具有显著不同于相应的未修饰TGF-β超家族异多聚体复合体的胞内半寿期的变体。例如,经改变的蛋白质可赋予对蛋白水解性降解或导致未修饰多肽破坏或否则失活的其它细胞过程更稳定或更不稳定。这类变体和编码它们的基因可用于通过调节多肽的半寿期来改变多肽复合体水平。例如,当诱导型表达系统的一部分可允许较严密控制细胞内的重组多肽复合体水平时,短半寿期可产生更短暂的生物作用。在Fc融合蛋白中,可在接头(如有的话)和/或Fc部分中进行突变以改变TGF-β超家族异多聚体复合体的一种或多种活性,包括例如免疫原性、半寿期和溶解度。

[0439] 可通过编码多肽文库的基因的简并文库产生组合文库,所述多肽各自包含可能的TGF-β超家族I型和/或II型受体序列的至少一部分。例如,可以酶的方法将合成寡核苷酸的混合物与基因序列连接,使得可能的TGF-β超家族I型和/或II型受体编码核苷酸序列的简并组可作为个别多肽表达,或者作为一组较大的融合蛋白表达(例如用于噬菌体展示)。

[0440] 存在许多可从简并寡核苷酸序列产生可能的同源物文库的方法。简并基因序列的化学合成可在自动DNA合成仪中进行,合成基因然后与用于表达的合适载体连接。简并寡核苷酸的合成是本领域众所周知的。参见例如Narang,SA(1983)Tetrahedron39:3;Itakura等(1981)Recombinant DNA,Proc.3rd Cleveland Sympos.Macromolecules,ed.AG Walton,Amsterdam:Elsevier pp273-289;Itakura等(1984)Annu.Rev.Biochem.53:323;Itakura等(1984)Science198:1056;Ike等(1983)Nucleic AcidRes.11:477。这类技术已应用于其它蛋白质的定向进化中。参见例如Scott等(1990)Science249:386-390;Roberts等(1992)

PNAS USA89:2429-2433;Devlin等(1990)Science249:404-406;Cwirla等(1990)PNASUSA87:6378-6382;以及美国专利号:5,223,409、5,198,346和5,096,815。

[0441] 或者,可利用其它诱变形式产生组合文库。例如,可采用以下诱变通过筛选,从文库中产生和分离TGF-β超家族异多聚体复合体:例如丙氨酸扫描诱变等[参见例如Ruf等(1994)Biochemistry33:1565-1572;Wang等(1994)J.Biol.Chem.269:3095-3099;Balint等(1993)Gene137:109-118;Grodberg等(1993)Eur.J.Biochem.218:597-601;Nagashima等(1993)J.Biol.Chem.268:2888-2892;Lowman等(1991)Biochemistry30:10832-10838;和Cunningham等(1989)Science244:1081-1085];通过接头扫描诱变[参见例如Gustin等(1993)Virology193:653-660;和Brown等(1992)Mol.Cell Biol.12:2644-2652;McKnight等(1982)Science232:316];通过饱和诱变[Meyers等(1986)Science232:613];通过PCR诱变[Leung等(1989)Method Cell Mol Biol1:11-19];或者通过随机诱变,包括化学诱变等[Miller等(1992)A Short Course in Bacterial Genetics,CSHL Press,Cold Spring Harbor,NY;以及Greener等(1994)Strategies in Mol Biol7:32-34]。接头扫描诱变,特别在组合背景下,是用于鉴定截短(生物活性)形式的TGF-β超家族I型和/或II型受体多肽的有吸引力的方法。

[0442] 本领域已知用于筛选通过点突变和截短制备的组合文库的基因产物和在这方面用于筛选具有某种性质的基因产物的cDNA文库的各种技术。这类技术一般可适用于快速筛选由本公开内容的TGF-β超家族异多聚体复合体的组合诱变所产生的基因文库。最广泛采用的筛选大型基因文库的技术通常包括将基因文库克隆至可复制的表达载体中,用所得载体文库转化合适的细胞,并在其中所需活性的检测有利于相对容易地分离编码其产物被检测的基因的载体的条件下,表达组合基因。优选的测定法包括TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)结合测定法和/或TGF-β超家族配体介导的细胞信号转导测定法。

[0443] 在某些实施方案中,除天然存在于TGF-β超家族I型和II型异多聚体复合体中的任何修饰以外,本发明的TGF-β超家族I型和II型异多聚体复合体还可包含翻译后修饰。这类修饰包括但不限于乙酰化、羧化、糖基化、磷酸化、脂化和酰化。因此,修饰的TGF-Β超家族I型和II型异多聚体复合体可含有非氨基酸成分,例如聚乙二醇、脂质、多糖或单糖和磷酸酯。可按照本文有关其它异多聚体复合体变体方面所述,测试这类非氨基酸成分对异多聚体复合体的功能性的作用。如果本公开内容的多肽在细胞中通过切割该多肽的新生形式产生,则翻译后加工对于蛋白质的正确折叠和/或功能也可能十分重要。对于这类翻译后活性,不同的细胞(例如CHO、HeLa、MDCK、293、WI38、NIH-3T3或HEK293)具有特定的细胞机器和特有机制,可选择不同的细胞以确保TGF-β超家族I型和/或II型受体多肽以及包含所述受体多肽的异多聚体的正确修饰和加工。

[0444] 在某些方面,本文公开的多肽可形成包含与至少一种II型受体多肽共价或非共价缔合的至少一种TGF-β超家族I型多肽的蛋白质复合体。优选本文公开的多肽形成异二聚体复合体,但还包括更高阶的异多聚体复合体(异多聚体),例如但不限于异三聚体、异四聚体

和其它的寡聚体结构(参见例如图1、2和15-17)。在一些实施方案中,本公开内容的TGF-β超家族I型和/或II型受体多肽包含至少一个多聚化结构域。如本文所公开的,术语"多聚化结构域"是指促进至少一个第一多肽和至少一个第二多肽间的共价或非共价相互作用的氨基酸或氨基酸的序列。本文公开的多肽可与多聚化结构域共价或非共价连接。优选多聚化结构域促进第一多肽(例如TGF-β超家族I型多肽)和第二多肽(例如TGF-β超家族II型多肽)间的相互作用以促进异多聚体形成(例如异二聚体形成),并任选阻碍或以别的方式不利于同多聚体形成(例如同二聚体形成),从而提高所需异多聚体的收率(参见例如图2)。

[0445] 可采用本领域已知的许多方法产生本公开内容的TGF-β超家族复合体。例如,非天然存在的二硫键可如下构建:通过在第一多肽(例如TGF-β超家族I型多肽)上用含有游离硫醇基的残基(例如半胱氨酸)置换天然存在的氨基酸,使得游离硫醇基与第二多肽(例如TGF-β超家族II型多肽)上的另一含有游离硫醇基的残基相互作用,从而在第一和第二多肽之间形成二硫键。相互作用以促进异多聚体形成的其它实例包括但不限于例如描述于Kjaergaard等,WO2007147901的离子相互作用;例如描述于Kannan等,U.S.8,592,562的静电引导作用(electrostatic steering effect);例如描述于Christensen等,U.S.20120302737的卷曲螺旋相互作用;例如描述于Pack&Plueckthun,(1992)Biochemistry31:1579-1584的亮氨酸拉链;以及例如描述于Pack等(1993)Bio/Technology11:1271-1277的螺旋-转角-螺旋基序。不同区段的连接可如下获得:经由例如通过化学交联、肽接头、二硫键等的共价结合,或例如通过抗生物素蛋白-生物素或亮氨酸拉链技术亲和相互作用。

[0446] 在某些方面,多聚化结构域可包含相互作用对的一个组分。在一些实施方案中,本文公开的多肽可形成包含与第二多肽共价或非共价缔合的第一多肽的蛋白质复合体,其中第一多肽包含TGF-β超家族I型多肽的氨基酸序列和相互作用对的第一成员的氨基酸序列;第二多肽包含TGF-β超家族II型多肽的氨基酸序列和相互作用对的第二成员的氨基酸序列。相互作用对可以是相互作用形成复合体、特别是异二聚体复合体的任何2个多肽序列,但是有效实施方案也可使用可形成同二聚体复合体的相互作用对。一个相互作用对的成员可与本文所述TGF-β超家族I型或II型多肽融合,包括例如与SEQ ID NO:14、15、124、126、171、172、413、414、463、464、18、19、136、138、173、174、421、422、465、466、22、23、115、117、175、176、407、408、467、468、26、27、83、84、104、106、177、178、403、404、469、470、30、31、87、88、139、141、179、180、423、424、471、472、34、35、91、92、142、144、181、182、425、426、473、474、38、39、301、302、305、306、309、310、313、112、114、183、184、405、406、475、476、9、10、11、118、120、151、152、409、410、451、452、1、2、3、4、5、6、100、102、153、154、401、402、453、454、46、47、71、72、121、123、155、156、411、412、455、456、50、51、75、76、79、80、133、135、161、162、419、420、457、458、42、43、67、68、127、129、130、132、157、158、159、160、415、416、417、418、459、460、461和462中任一个的序列有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列的多肽序列、基本由其组成或由其组成。可选择相互作用对以赋予改进的性质/活性例如延长的血清半寿期,或作为另一部分与之连接以提供改进的性质/活性的衔接物的作用。例如,聚乙二醇部分可与相互作用对的一个或两个组成分连接以提供改进的性质/活性例如改进的血清半寿期。

[0447] 相互作用对的第一或第二成员可以是不对称对,意味着所述对的成员优先彼此缔

合而非自身缔合。因此，不对称相互作用对的第一和第二成员可缔合形成异二聚体复合体(参见例如图2)。或者，相互作用对可为不受引导的，意味着所述对的成员可彼此缔合或自身缔合而无实质性偏好，因此可具有相同或不同的氨基酸序列。因此，不受引导相互作用对的第一和第二成员可缔合形成同二聚体复合体或异二聚体复合体。任选相互作用对(例如不对称对或不受引导相互作用对)的第一成员与相互作用对的第二成员共价缔合。任选相互作用对(例如不对称对或不受引导相互作用对)的第一成员与相互作用对的第二成员非共价缔合。

[0448] 作为具体实例，本公开内容提供融合蛋白，其包含与包含免疫球蛋白的恒定结构域(例如免疫球蛋白的CH1、CH2或CH3结构域或Fc结构域)的多肽融合的TGF-β超家族I型或II型多肽。本文提供衍生自人IgG1、IgG2、IgG3和IgG4的Fc结构域。已知降低CDC或ADCC活性的其它突变，总的来说，这些变体的任一个都包括在本公开内容中，并且可用作本公开内容的异多聚体复合体的有利组分。任选SEQ ID NO:208的IgG1Fc结构域在例如Asp-265、Lys-322和Asn-434(按照相应的全长IgG1编号)等残基上具有一个或多个突变。在某些情况下，具有这些突变(例如Asp-265突变)的一个或多个的突变型Fc结构域相对于野生型Fc结构域与Fcγ受体结合的能力降低。在其它情况下，具有这些突变(和Asn-434突变)的一个或多个的突变型Fc结构域相对于野生型Fc结构域与MHCI类相关Fc-受体(FcRN)结合的能力降低。

[0449] 可用于人IgG1的Fc部分(G1Fc)的天然氨基酸序列的实例见下(SEQ ID NO:208)。点下划线表示铰链区，实线下划线表示含天然存在的变体的位置。一方面，本公开内容提供包含与SEQ ID NO:208有70％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列、由其组成或基本由其组成的多肽。G1Fc中天然存在的变体可包括E134D和M136L，按照用于SEQ ID NO:208的编号系统(参见Uniprot P01857)。

[0450]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 208)
```

[0451] 可用于人IgG2(G2Fc)的Fc部分的天然氨基酸序列的实例见下(SEQ ID NO:209)。点下划线表示铰链区，双下划线表示其中在序列(按照UniProtP01859)中冲突的数据库的位置。一方面，本公开内容提供包含与SEQ ID NO:209有70％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列、由其组成或基本由其组成的多肽。

[0452]

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

[0453]

[0454] 可用人IgG3于的Fc部分的氨基酸序列(G3Fc)的2个实例见下。G3Fc中的铰链区长可多达其它Fc链的4倍，并含有3个相同的15-残基区段前面接相似的17-残基区段。下面显

示的第一G3Fc序列(SEQ ID NO:210)含有由单个15-残基区段组成的短铰链区,而第二G3Fc序列(SEQ ID NO:211)含有全长铰链区。按照UniProt P01859,在每种情况下,点下划线表示铰链区,实线下划线表示含天然存在的变体的位置。一方面,本公开内容提供多肽包含与SEQ ID NO:210和211有70%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列、由其组成或基本由其组成。

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

[0455]

```
  1  ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
 51  SCDTPPPCPR CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
101  EDPEVQFKWY VDGVEVHNAK TKPREEQYNS TFRVVSVLTV LHQDWLNGKE
151  YKCKVSNKAL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL
201  VKGFYPSDIA VEWESSGQPE NNYNTTPPML DSDGSFFLYS KLTVDKSRWQ
251  QGNIFSCSVM HEALHNRFTQ KSLSLSPGK        (SEQ ID NO: 211)
```

[0456] G3Fc中天然存在的变体(例如,参见Uniprot P01860)当转换成用于SEQ ID NO:210的编号系统时包括E68Q、P76L、E79Q、Y81F、D97N、N100D、T124A、S169N、S169del、F221Y,本公开内容提供包含含有这些变化的一个或多个的G3Fc结构域的融合蛋白。另外,人免疫球蛋白IgG3基因(IGHG3)显示特征在于不同铰链长度的结构多态性[参见Uniprot P01859]。具体而言,变体WIS缺乏大部分V区和全部CH1区。除了正常存在于铰链区的11位以外,在7位具有额外的链间二硫键。变体ZUC缺乏大部分的V区、全部CH1区和部分铰链。变体OMM可呈现等位基因形式或另一种γ链亚类。本公开内容提供包含含有这些变体的一个或多个的G3Fc结构域的其它融合蛋白。

[0457] 可用于人IgG4(G4Fc)的Fc部分的天然氨基酸序列的实例见下(SEQ ID NO:212)。点下划线表示铰链区。一方面,本公开内容提供包含与SEQ ID NO:212有70%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列、由其组成或基本由其组成的多肽。

```
  1  ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ
 51  EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE
101  YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL
151  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ
201  EGNVFSCSVM HEALHNHYTQ KSLSLSLGK(SEQ ID NO: 212)
```

[0458]

[0459] 本文提供相对于G1Fc序列(SEQ ID NO:208)在Fc结构域中有多个工程改造的突变,且G2Fc、G3Fc和G4Fc中的类似突变可来源于与图5中G1Fc的比对。由于不等铰链长度所致,基于同种型比对的类似的Fc位置(图5)在SEQ ID NO:208、209、210和212中具有不同的氨基酸数目。还可以认识到,当如Uniprot数据库中一样编号包括整个IgG1重链恒定结构域

(由C_H1、铰链、C_H2和C_H3区组成)时,可通过与相同位置不同的编号来鉴定由铰链、C_H2和C_H3区组成的免疫球蛋白序列中指定的氨基酸位置(例如SEQ ID NO:208、209、210、211或212)。例如,人G1Fc序列(SEQ ID NO:208)、人IgG1重链恒定结构域(Uniprot P01857)和人IgG1重链中所选C_H3位置间的对应如下。

[0460]

不同编号系统中 C_H3 位置的对应		
G1Fc (编号始于铰链区的第一苏氨酸)	IgG1 重链恒定结构域 (编号始于 C_H1)	IgG1 重链 (Kabat 等的 EU 编号方案, 1991*)
Y127	Y232	Y349
S132	S237	S354

[0461]

E134	E239	E356
T144	T249	T366
L146	L251	L368
K170	K275	K392
D177	D282	D399
Y185	Y290	Y407
K187	K292	K409
* Kabat 等(编辑) 1991;pp. 688-696,载于 *Sequences of Proteins of Immunological Interest*, 5th ed., Vol. 1, NIH, Bethesda, MD。		

[0462] 在自单细胞系大规模生产基于不对称免疫球蛋白的蛋白质中出现的问题称为“链缔合问题(chain association issue)”。正如主要在双特异性抗体产生中所遇到的,链缔合问题关心当在单细胞系中产生不同的重链和/或轻链时,在固有导致的多种组合中有效产生所需多链蛋白质的挑战[参见例如,Klein等(2012)mAbs4:653-663]。当2条不同的重链和2条不同的轻链在同一细胞中产生的,这个问题最为严重,在这种情况下,共有16种可能的链组合(虽然这些中的一些是相同的),然而通常只有一种是需要的。尽管如此,相同原理说明了仅掺入2条不同的(不对称)重链的所需多链融合蛋白的收率降低。

[0463] 在单细胞系中提高所需含Fc的融合多肽链的配对从而以可接受的收率产生优选的不对称融合蛋白的各种方法是本领域已知的[参见例如,Klein等(2012)mAbs4:653-663;以及Spiess等(2015)Molecular Immunology67(2A):95-106]。获得所需含Fc链的配对的方法包括但不限于基于带电荷的配对(静电引导)、“孔中结(knob-into-hole)”空间配对、SEEDbody配对和基于亮氨酸拉链的配对。参见例如Ridgway等(1996)Protein Eng9:617-621;Merchant等(1998)Nat Biotech16:677-681;Davis等(2010)Protein Eng Des Sel23:195-202;Gunasekaran等(2010);285:19637-19646;Wranik等(2012)JBiolChem287:43331-43339;US5932448;WO1993/011162、WO2009/089004和WO2011/034605。如本文所述,这些方法可用来产生包含TGF-β超家族I型和II型受体多肽、至少两种不同的TGF-β超家族I型受体

多肽和至少两种不同的TGF-β超家族II型受体多肽的异二聚体。参见图15-17。

[0464] 例如,可籍以促进特定多肽间的相互作用的一种方法是通过例如描述于Arathoon等,U.S.7,183,076和Carter等,U.S.5,731,168的方法对凹处凸起(protuberance-into-cavity)(孔中结)互补区进行改造。“凸起”通过将来自第一多肽(例如第一相互作用对)界面的小氨基酸侧链用较大的侧链(例如酪氨酸或色氨酸)置换来构建。与凸起相同或类似大小的互补“凹处”任选在第二多肽(例如第二相互作用对)界面上通过将大氨基酸侧链用较小的侧链(例如丙氨酸或苏氨酸)置换产生。在适当定位和确定尺寸的凸起或凹处存在于第一或第二多肽任一个的界面上时,只需要分别对邻近界面上的相应凹处或凸起进行改造。

[0465] 在中性pH(7.0)下,天冬氨酸和谷氨酸带有负电荷,赖氨酸、精氨酸和组氨酸带正电荷。这些带电荷的残基可用来促进异二聚体形成,同时阻碍同二聚体形成。吸引的相互作用发生在相反电荷之间,而排斥的相互作用发生在相同电荷之间。一方面,通过对带电荷的界面残基进行位点定向诱变,本文公开的蛋白质复合体利用有吸引的相互作用以促进异多聚体形成(例如异二聚体形成),并任选利用排斥的相互作用以阻碍同二聚体形成(例如同二聚体形成)。

[0466] 例如,IgG1CH3结构域界面包含参与结构域-结构域相互作用的4个独特的带电荷残基对:Asp356-Lys439’、Glu357-Lys370’、Lys392-Asp399’和Asp399-Lys409’[第二链的残基编号用(’)表示]。应注意,本文所用的标明IgG1 CH3结构域中的残基的编号方案与Kabat的EU编号方案一致。由于CH3-CH3结构域相互作用中存在的2倍对称性,因此各个单独的相互作用将在结构(例如Asp-399-Lys409’和Lys409-Asp399’)中出现两次。在野生型序列中,K409-D399’有利于异二聚体和同二聚体两者形成。改变第一链中的电荷极性(例如K409E;正电荷至负电荷)的单突变对第一链同二聚体的形成产生不利的相互作用。不利的相互作用因发生在相同电荷(负-负;K409E-D399’和D399-K409E’)间的排斥相互作用而产生。改变第二链中电荷极性的类似突变(D399K’;负-正)对第二链同二聚体形成产生不利的相互作用(K409’-D399K’和D399K-K409’)。但同时,这两个突变(K409E和D399K’)对异二聚体形成产生不利的相互作用(K409E-D399K’和D399-K409’)。

[0467] 通过可与或不与第二链中带相反电荷的残基(包括例如Arg355和Lys360)配对的其它带电荷残基的突变,可进一步增强对异二聚体形成和同二聚体阻碍的静电引导作用。下表列出可单独或组合使用的可能的电荷变化突变以提高本文公开的多肽复合体的异多聚体形成。

[0468]

成对带电荷残基突变提高异二聚体形成的实例			
第一链 中的位置	第一链 中的突变	第二链 中的相互作用位置	第二链 中的相应突变
Lys409	Asp 或 Glu	Asp399’	Lys、Arg 或 His
Lys392	Asp 或 Glu	Asp399’	Lys、Arg 或 His
Lys439	Asp 或 Glu	Asp356’	Lys、Arg 或 His
Lys370	Asp 或 Glu	Glu357’	Lys、Arg 或 His
Asp399	Lys、Arg 或 His	Lys409’	Asp 或 Glu
Asp399	Lys、Arg 或 His	Lys392’	Asp 或 Glu
Asp356	Lys、Arg 或 His	Lys439’	Asp 或 Glu
Glu357	Lys、Arg 或 His	Lys370’	Asp 或 Glu

[0469] 在一些实施方案中，构成本申请的融合蛋白中的CH3-CH3界面的一个或多个残基用带电荷的氨基酸置换，使得相互作用变成静电上不利的。例如，界面中的带正电荷的氨基酸(例如赖氨酸、精氨酸或组氨酸)用带负电荷的氨基酸(例如天冬氨酸或谷氨酸)置换。或者，与前述取代组合，将界面中带负电荷的氨基酸用带正电荷的氨基酸置换。在某些实施方案中，氨基酸用具有所需电荷性质的非天然存在的氨基酸置换。应注意，使带负电荷的残基(Asp或Glu)突变为His将导致侧链体积增加，这可能引起空间问题。此外，His质子供体形式和受体形式取决于局部环境。应根据设计策略考虑这些问题。因为界面残基在人和小鼠IgG亚类中是高度保守的，本文公开的静电引导作用可适用于人和小鼠IgG1、IgG2、IgG3和IgG4。这个策略还可延伸至在CH3结构域界面修饰不带电荷的残基成为带电荷的残基。

[0470] 一方面，本公开内容提供使用经改造在电荷配对(静电引导)基础上互补的Fc序列的含不对称Fc的多肽链的所需配对。在有或没有任选接头的情况下，具有静电互补性的一对Fc序列之一可任意与构建体的TGF-β超家族I型或II型受体多肽融合，产生TGF-β超家族I型或II型受体融合多肽。这个单链连同与第一Fc互补的Fc序列一起在精选细胞中共表达以利于所需多链构建体(例如TGF-β超家族异聚体复合体)的产生。在基于静电引导的这个实例中，SEQ ID NO:200[人G1Fc(E134K/D177K)]和SEQ ID NO:201[人G1Fc(K170D/K187D)]是互补Fc序列的实例，其中经改造的氨基酸取代用双下划线表示，构建体的TGF-β超家族I型或II型受体多肽可与SEQ ID NO:200或SEQ ID NO:201任一个但非两个融合。鉴于天然hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc间的高度氨基酸序列同一性，应认识到hG2Fc、hG3Fc或hG4Fc中相应位置的氨基酸取代(参见图5)将产生互补Fc对，这可代替下面的互补hG1Fc对(SEQ ID NO:200和201)使用。

[0471]

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

[0472] 一方面，本公开内容提供使用针对空间互补性改造的Fc序列的含不对称Fc的多肽链的所需配对。一方面，本公开内容提供孔中结配对作为空间互补性的实例。在有或没有任选接头的情况下，具有空间互补性的一对Fc序列之一可任意与构建体的TGF-β超家族I型或II型多肽融合，产生TGF-β超家族I型或II型融合多肽。这个单链连同与第一Fc互补的Fc序列一起在精选细胞中共表达以利于所需多链构建体的产生。在基于孔中结配对的这个实例中，SEQ ID NO:202[人G1Fc(T144Y)]和SEQ ID NO:203[人G1Fc(Y185T)]是互补Fc序列的实例，其中经改造的氨基酸取代用双下划线表示，且构建体的TGF-β超家族I型或II型多肽可与SEQ ID NO:202或SEQ ID NO:203的任一个但非两个融合。鉴于天然hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc之间的高度氨基酸序列同一性，应认识到hG2Fc、hG3Fc或hG4Fc中相应位置的氨基酸取代(参见图5)将产生互补Fc对，这可代替下面的互补hG1Fc对使用(SEQ ID NO:202和203)。

[0473]

```
  1   THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51   VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101   VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLYCLVKGF
151   YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201   FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 202)
  1   THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51   VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101   VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151   YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLTSKLTV DKSRWQQGNV
201   FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 203)
```

[0474] 基于与经改造的二硫键组合的孔中结配对的Fc互补性的实例公开于SEQ ID NO:204[hG1Fc(S132C/T144W)]和SEQ ID NO:205[hG1Fc(Y127C/T144S/L146A/Y185V)]。这些序列中经改造的氨基酸取代用双下划线表示，且构建体的TGF-β超家族I型或II型多肽可与SEQ ID NO:204或SEQ ID NO:205的任一个但非两个融合。鉴于天然hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc之间的高度氨基酸序列同一性，应认识到hG2Fc、hG3Fc或hG4Fc中相应位置的氨基酸取代(参见图5)将产生互补Fc对，这可代替下面的互补hG1Fc对使用(SEQ ID NO:204和205)。

[0475]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 204)
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSREEMTKNQ VSLSCAVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 205)
```

[0477] 一方面，本公开内容提供使用经改造以产生人IgG的交错β-链区段和IgAC$_H$3结构域的Fc序列的含不对称Fc的多肽链的所需配对。所述方法包括使用允许SEEDbody融合蛋白形成的链交换改造的结构域(SEED)C$_H$3异二聚体[参见例如Davis等(2010)Protein Eng Design Sel23:195-202]。在有或没有任选接头的情况下，具有SEEDbody互补性的Fc序列对之一可任意与构建体的TGF-β超家族I型或II型多肽融合，产生TGF-β超家族I型或II型融合多肽。这个单链连同与第一Fc互补的Fc序列一起在精选细胞中共表达以利于所需多链构建体的产生。在基于SEEDbody(Sb)配对的这个实例中，SEQ ID NO:206[hG1Fc(Sb$_{AG}$)]和SEQ ID NO:207[hG1Fc(Sb$_{GA}$)]是互补IgG Fc序列的实例，其中来自IgA Fc的经改造的氨基酸取代用双下划线表示，且构建体的TGF-β超家族I型或II型多肽可与SEQ ID NO:206或SEQ ID NO:207任一个但非两个融合。鉴于天然hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc之间的高度氨基酸序列同一性，应认识到hG1Fc、hG2Fc、hG3Fc或hG4Fc中相应位置的氨基酸取代(参见图5)将产生Fc单体，其可用于下面的互补IgG-IgA对(SEQ ID NO:206和207)。

[0478]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PFRPEVHLLP PSREEMTKNQ VSLTCLARGF
151  YPKDIAVEWE SNGQPENNYK TTPSRQEPSQGTTTFAVTSK LTVDKSRWQQ
201  GNVFSCSVMH EALHNHYTQK TISLSPGK       (SEQ ID NO: 206)
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PPSEELALNELVTLTCLVKG
151  FYPSDIAVEW ESNGQELPREKYLTWAPVLD SDGSFFLYSI LRVAAEDWKK
201  GDTFSCSVMH EALHNHYTQK SLDRSPGK       (SEQ ID NO: 207)
```

[0479] 一方面，本公开内容提供具有在Fc C$_H$3结构域C端连接的可切割亮氨酸拉链结构域的含不对称Fc多肽链的所需配对。亮氨酸拉链的连接足以引起异二聚体抗体重链优先装配。参见例如Wranik等(2012)J Biol Chem287:43331-43339。如本文所公开的，在有或没有任选接头的情况下，与亮氨酸拉链形成链连接的Fc序列对之一可任意与构建体的TGF-β超家族I型或II型多肽融合，产生TGF-β超家族I型或II型融合多肽。这个单链可连同与互补亮氨酸拉链形成链连接的Fc序列一起在精选细胞中共表达以利于所需多链构建体的产生。在纯化后，构建体用细菌内切蛋白酶Lys-C蛋白水解消化可释放亮氨酸拉链结构域，产生其结

构与天然Fc的相同的Fc构建体。在基于亮氨酸拉链配对的这个实例中，SEQ ID NO:213[hG1Fc-Ap1(酸性)]和SEQ ID NO:214[hG1Fc-Bp1(碱性)]是互补IgG Fc序列的实例，其中经改造的互补亮氨酸拉链序列用下划线表示，且构建体的TGF-β超家族I型或II型多肽可与SEQ ID NO:213或SEQ ID NO:214的任一个但非两个融合。鉴于天然hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc之间的高度氨基酸序列同一性，可了解在有或没有任选接头的情况下，与hG1Fc、hG2Fc、hG3Fc或hG4Fc连接的亮氨酸拉链形成序列(参见图5)将产生Fc单体，其可用于下面的互补亮氨酸拉链形成对(SEQ ID NO:213和214)。

[0480]

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

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

[0481] 如上所述，本领域已知在单细胞系中增加含Fc的融合多肽链的所需配对从而以可接受的收率产生优选的不对称融合蛋白的各种方法[Klein等(2012)mAbs4:653-663；和Spiess等(2015)Molecular Immunology67(2A):95-106]。另外，可使用包含TGF-β超家族I型或TGF-β超家族II型多肽的重链和轻链融合蛋白的组合，产生本文所述异多聚体。例如，在一些实施方案中，TGF-β超家族I型受体多肽可在有或没有接头结构域的情况下与包含C_H1结构域的至少一部分的免疫球蛋白重链(IgG1、IgG2、IgG3、IgG4、IgM、IgA1或IgA2)融合。同样，TGF-β超家族II型受体多肽可在有或没有接头结构域的情况下与包含轻链恒定结构域(C_L)的至少一部分的免疫球蛋白轻链(κ或λ)融合。在备选的实施方案中，TGF-β超家族I型受体多肽可在有或没有接头结构域的情况下与包含C_H1结构域的至少一部分的免疫球蛋白重链(IgG1、IgG2、IgG3、IgG4、IgM、IgA1或IgA2)融合，且TGF-β超家族II型受体多肽可在有或没有接头结构域的情况下与包含轻链恒定结构域(C_L)的至少一部分的免疫球蛋白轻链(κ或λ)融合。该设计利用重链与轻链异二聚化的天然能力。具体地讲，重链和轻链的异源二聚化发生在C_H1与C_L之间，这一般通过使2个结构域经由二硫键共价连接来稳定。使用全长重链或包含铰链区的重链的至少一部分的构建体，可产生包含两条“轻链”和两条“重链”的抗体样分子。参见图16。该设计的潜在优势是它可更接近地模拟天然存在的TGF-β超家族I型受体多肽-配体-TGF-β超家族II型受体多肽复合体，并可对配体显示比相应的单一异二聚体高的亲和力。在一些实施方案中，该设计可通过掺入不同的重链截短物包括例如包含C_H1结构域和铰链结构域的一些或全部的截短物(产生$F(ab')_2$样分子)以及只包含C_H1结构域或其片段的截短物(产生Fab样分子)来改进。参见图16G。设计这类异多聚体构建体的各种方法描述于US2009/0010879、Klein等[(2012)mAbs4:653-663]和Spiess等[(2015)

Molecular Immunology67(2A):95-106],其内容通过引用以其整体结合到本文中。

[0482] 在一些实施方案中,需要产生这样的抗体样异二聚体:其包含含有TGF-β超家族I型受体多肽-C_L:TGF-β超家族II型受体多肽-C_H1异二聚体对的复合体的至少一支和包含TGF-β超家族II型受体多肽-C_L:TGF-β超家族I型受体多肽r-C_H1异二聚体对的至少第二支。参见例如图16B。这类异二聚体复合体可采用例如重链和轻链不对称配对技术的组合产生[Spiess等(2015)Molecular Immunology67(2A):95-106]。例如在CrossMab技术[Schaefer等(2011).Proc.Natl.Acad.Sci.U.S.A.108:11187-11192]中,轻链错配使用结构域交叉(domain crossover)和使用孔中结异二聚化的重链来克服[Merchant等(1998)Nat.Biotechnol.16:677-681]。对于结构域交叉,可变结构域或恒定结构域可在轻链和重链之间交换,以产生驱动关联轻链配对同时保持可变结构域的结构和功能完整性的2个不对称Fab臂[Fenn等(2013)PLoS ONE8:e61953]。用于克服轻链错配的替代方法设计了具有正交的Fab界面的重链和轻链[Lewis(2014)Nat.Biotechnol.32:191-198]。这通过计算建模[Das等(2008)Annu.Rev.Biochem.77:363-382]与X射线晶体学的组合实现以鉴定V_H/V_L和C_H1/C_L界面上的突变。对于采用该方法产生的异二聚体,可能需要将突变改造进入V_H/V_L和C_H1/C_L界面两者中以使重链/轻链错配最小化。设计的正交Fab界面可与重链异二聚化策略联用以在单宿主细胞中促进有效的IgG产生。静电引导也可用来产生正交Fab界面以促进这类异二聚体的构建。肽接头可用于确保呈称为“LUZ-Y”形式的轻链和重链的关联配对[Wranik等(2012)J.Biol.Chem.287:43331-43339],其中重链异二聚化使用随后可通过体外蛋白酶解除去的亮氨酸拉链实现。

[0483] 或者,异多聚体可包含本文所述的一个或多个单链配体阱,任选其可与一种或多种TGF-β超家族I型受体多肽或TGF-β超家族I型受体多肽以及其它的TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽单链配体阱共价或非共价缔合[US2011/0236309和US2009/0010879]。参见图12。如本文所述,单链配体阱不需要与任何多聚化结构域(例如卷曲螺旋Fc结构域)融合成为多价的。一般而言,本公开内容的单链配体阱包含至少一种TGF-β超家族I型受体多肽结构域和一种TGF-β超家族I型受体多肽结构域。TGF-β超家族I型受体多肽和TGF-β超家族I型受体多肽结构域,在本文一般称为结合结构域(BD),任选可通过接头区连接。

[0484] 例如,一方面,本公开内容提供包含具有下列结构的多肽的异多聚体:

[0485] $(<BD1>\text{-接头1})_k\text{-}[<BD2>\text{-接头2-}\{<BD3>\text{-接头3}\}_f]_n\text{-}(<BD4>)_m\text{-}(\text{接头4-}BD5>_d)_h$

[0486] 其中:n和h独立地大于或等于1;d、f、m和k独立地大于或等于零;BD1、BD2、BD3、BD4和BD5独立地为TGF-β超家族I型受体多肽或TGF-β超家族II型受体多肽结构域,其中BD1、BD2、BD3和BD4的至少一个是TGF-β超家族I型受体多肽结构域,且其中BD1、BD2、BD3和BD4的至少一个是TGF-β超家族II型受体多肽结构域,接头1、接头2、接头3和接头4独立地为大于或等于零。在一些实施方案中,TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽单链阱包含至少两种不同的TGF-β超家族I型受体多肽。在一些实施方案中,TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽单链阱包含至少两种不同的TGF-β超家族II型受体多肽。在一些实施方案中,TGF-β超家族I型受体多肽:TGF-β超家族I型受体多肽单链阱包含至少两种不同的接头。根据对d、f、h、k、m和n选择的值,异多聚体结构可包含呈不同组合的大量重复单位或可以是相对简单的结构。

[0487] 另一方面，本公开内容提供包含具有下列结构的多肽的异多聚体：

[0488] <BD1>-接头1-<BD2>

[0489] 又一方面，本公开内容提供包含具有下列结构的多肽的异多聚体：

[0490] <BD1>-(接头2-<BD2>)$_n$

[0491] 其中n大于或等于1。

[0492] 本发明的另一方面提供包含具有下列结构的多肽的异多聚体：

[0493] (<BD1>-接头1-<BD1>)$_f$-接头2-(<BD2>-接头3-<BD3>)$_g$

[0494] 其中f和g大于或等于1。

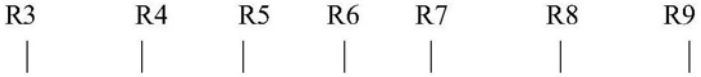
[0495] 在其中BD2和BD3是相同且f和g是相同的数值的实施方案中，这导致以接头2为中心的基本镜面对称的结构，以接头的差异为准。在其中BD2不同于BD3和/或其中f和g为不同的数值的情况下，将产生不同的结构。根据本文的公开内容和本领域的知识选择合适的结合结构域、接头和重复频率属于本领域普通技术人员的能力之内。图18中以示意图显示本公开内容的这类单链配体阱的具体的非限制性实例。

[0496] 接头(1、2、3和4)可相同或不同。接头区提供完全不同于TGF-β超家族I型受体多肽和TGF-β超家族II型受体多肽的结构性配体结合结构域的区段，因此可用于与辅助分子(例如可用于提高稳定性的分子，例如聚乙二醇化部分)缀合而不必对结合结构域进行化学修饰。接头可包括非结构性氨基酸序列，其可与目标配体的受体或TGF-β超家族的另一受体的胞外部分天然非结构性区的序列相同或来源于对其的保守修饰。在其它情况下，这类接头在组成或来源上可能完全是人工的，但可含有经选择的氨基酸以提供当与目标配体接近时遇到静电或空间阻碍难题的可能性低的非结构性柔性接头。以下接头长度可被为是可接受的：当允许位于接头N端和C端各端的结合结构域与其天然配体上的其天然结合部位结合，使得其两个结合结构域如此结合，配体以比通过仅结合结构域之一的结合高的亲和力结合。在某些情况下，选择天然或人工来源的接头中氨基酸残基的数目等于或大于与TGF-β超家族I型受体多肽和/或TGF-β超家族II型受体多肽配体上的两个结合部位同时(桥接)结合的最小所需距离。例如，虽不希望以任何方式予以限制，但是接头长度可介于约1-10个氨基酸、10-20个氨基酸、18-80个氨基酸、25-60个氨基酸、35-45个氨基酸或任何其它合适的长度。

[0497] 接头可经设计以利于多肽的纯化。所选的精确的纯化方案将确定需要什么样的修饰，例如且虽然不希望是限制性的，但考虑加入纯化“标签”例如His标签；在其它实例中，接头可包括有利于运货分子或辅助分子分子加入的区域。当这类添加影响接头的非结构性性质或引入可能的静电或空间顾虑时，可进行接头长度的适当增加以确保两个结合结构域能够结合其配体上相应的部位。根据本文的方法和教导内容，本领域技术人员可按常规进行所述决定。

[0498] 另外，本设计允许其它运货分子(例如成像剂像荧光分子)、毒素等的连接。例如且虽不希望以任何方式予以限制，但是可修饰单链多肽以加入一个或多个运货分子和/或辅助分子(本文统称为R1、R2、R3、R4等)：

[0499]



R1-(<BD1>-接头 1)$_k$-[<BD2>-接头 2-{<BD3>-接头 3}$_f$]$_n$-(<BD4>)$_m$-(接头 4-BD5>$_d$)$_h$-R2

[0500] 在不限制可获得的R取代基的通用性的情况下,R1、R2、R3、R4、R5、R6、R7、R8、R9可以存在或不存在;当存在时,它们可相同或不同,并且可独立地为以下的一种或多种:用于靶向例如但不限于例如抗体片段(例如单链Fv)和/或单一结构域抗体(sdAb)的融合蛋白;放射疗法和/或成像剂,例如但不限于放射性核素(例如^{123}I、^{111}In、^{18}F、^{64}C、^{68}Y、^{124}I、^{131}I、^{90}Y、^{177}Lu、^{57}Cu、^{213}Bi、^{211}At)、荧光染料(例如AlexaFluor、Cy染料)和/或荧光蛋白标签(例如GFP、DsRed);用于化学疗法的细胞素剂,例如但不限于多柔比星、卡奇霉素、类美登素衍生物(例如DM1、DM4)、毒素(例如截短假单胞菌属(Pseudomonas)内毒素A、白喉毒素);基于纳米粒的载体,例如但不限于聚乙二醇(PEG)、与药物缀合的聚合物、纳米载体或成像剂(例如聚合物N-(2-羟基丙基)甲基丙烯酰胺(HPMA)、谷氨酸、PEG、葡聚糖);药物(例如但不限于多柔比星、喜树碱、紫杉醇、palatinate);纳米载体,例如但不限于纳米壳(nanoshell)或脂质体;成像剂,例如但不限于Supermagnetic Iron Oxide(SPIO);树枝状聚体(dendrimer);和/或用于配体纯化、浓缩或螯合的固相支持体(例如纳米粒、惰性树脂、合适的二氧化硅支持体)。

[0501] 一般而言,在所有可能的位置上具有运货或辅助分子不会是优选的,因为这可能引起空间或静电难题。然而,可根据本文的公开内容通过在结合结构域间模化接头并进行分子动力学模拟,按常规确定将运货或辅助分子加入任何指定的结构的一个或多个位置上的作用,以使分子力学能充分降低到最低并降低接头和本文教导的TGF-β超家族I型受体多肽和TGF-β超家族II型受体多肽间的空间和静电不相容性。

[0502] 可能优选的是将运货或辅助分子加至作用剂的接头部分而不是结合结构域,以降低结合功能中干扰的可能性。然而,加至结合结构域是可能的,在某些情况下可能是需要的,并且这种加入的作用可按本文所述通过模化结合剂和具有所建议的添加的接头,提前按常规确定。

[0503] 缀合方法可使用通过常见的反应基团(例如伯胺、琥珀酰亚胺基(NHS)酯和巯基反应基团)能够缀合的商用试剂盒进行。一些非限制性实例为:Alexa Fluor488蛋白标记试剂盒(Molecular Probes,Invitrogen检测技术)和聚乙二醇化试剂盒(Pierce Biotechnology Inc.)。

[0504] 在某些方面,TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽单链阱可与一种或多种TGF-β超家族I型受体多肽或TGF-β超家族II型受体多肽以及其它的TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽单链配体阱共价或非共价缔合形成更高阶的异多聚体,这可按照本文所述方法使用。参见例如图19。例如,TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽单链配体阱可另包含本文所述的多聚化结构域。在一些实施方案中,TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽单链配体阱包含Ig免疫球蛋白的恒定结构域。可选择所述免疫球蛋白恒定结构域以促进包含至少一种单链TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽阱的对称或不对称复合体。

[0505] 在某些方面,TGF-β超家族I型受体多肽:TGF-β超家族II型受体多肽单链阱或所述阱的组合可用作TGF-β超家族拮抗剂治疗或预防本文所述TGF-β超家族病症或疾病(例如肾病和/或代谢紊乱或病况)。

[0506] 要了解,融合蛋白(例如免疫球蛋白Fc融合蛋白)的不同组分可以与所需功能性一致的任何方式排布。例如,TGF-β超家族I型和/或II型受体多肽结构域可被置于异源结构域

的C端，或者，异源结构域可被置于TGF-β超家族I型和/或II型受体多肽结构域的C端。TGF-β超家族I型和/或II型受体多肽结构域和异源结构域不必与融合蛋白接近，且其它的结构域或氨基酸序列可包括在任一结构域的C端或N端或在结构域之间。

[0507] 例如，TGF-β超家族I型和/或II型受体融合蛋白可包含式A-B-C列出的氨基酸序列。B部分相当于TGF-β超家族I型和/或II型受体多肽结构域。A和C部分可独立地为0、1或不止一个氨基酸，且A和C部分两者当存在时与B是异源的。A和/或C部分可通过接头序列与B部分连接。接头可富含甘氨酸(例如2-10、2-5、2-4、2-3个甘氨酸残基)或甘氨酸和脯氨酸残基，并且可含有例如苏氨酸/丝氨酸和甘氨酸的单一序列或苏氨酸/丝氨酸和/或甘氨酸的重复序列，例如GGG(SEQ ID NO:58)、GGGG(SEQ ID NO:59)、TGGGG(SEQ ID NO:60)、SGGGG(SEQ ID NO:61)、TGGG(SEQ ID NO:62)或SGGG(SEQ ID NO:63)单一序列或重复序列。在某些实施方案中，TGF-β超家族I型和/或II型受体融合蛋白包含式A-B-C所列的氨基酸序列，其中A是前导(信号)序列，B由TGF-β超家族I型和/或II型受体多肽结构域组成，C是这样的多肽部分，其提高体内稳定性、体内半寿期、摄取/给药、组织定位或分布、蛋白质复合体的形成和/或纯化的一种或多种。在某些实施方案中，TGF-β超家族I型和/或II型受体融合蛋白包式A-B-C所列的含氨基酸序列，其中A是TPA前导序列，B由TGF-β超家族I型和/或II型受体多肽结构域组成，C是免疫球蛋白Fc结构域。优选的融合蛋白包含SEQ ID NO:100、102、104、106、112、114、115、117、118、120、121、123、124、126、127、129、401、402、403、404、405、406、407、408、409、410、411、412、413、414、415和416的任一个列出的氨基酸序列。

[0508] 在一些实施方案中，本公开内容的TGF-β超家族受体异多聚体复合体另包含一种或多种异源部分(结构域)以赋予所需性质。例如一些融合结构域特别可用于通过亲和层析法分离融合蛋白。这类融合结构域的众所周知的实例包括但不限于聚组氨酸、Glu-Glu、谷胱甘肽S转移酶(GST)、硫氧还蛋白、A蛋白、G蛋白、免疫球蛋白重链恒定区(Fc)、麦芽糖结合蛋白(MBP)或人血清白蛋白。可选择融合结构域以提供所必需的性质。为了亲和纯化的目的，使用用于亲和层析法的相关基质，例如谷胱甘肽缀合树脂、淀粉酶缀合树脂和镍缀合树脂或钴缀合树脂。这类基质的许多种可以“试剂盒”形式获得，例如Pharmacia GST纯化系统和可与(HIS_6)融合配偶体一起使用的Q IAexpress™系统(Qiagen)。作为另一个实例，可选择融合结构域以促进配体阱多肽的检测。这类检测结构域的实例包括各种荧光蛋白(例如GFP)以及“表位标签”，其通常是短肽序列，可获得针对其的特异性抗体。易获得其特异性单克隆抗体的众所周知的表位标签包括FLAG、流感病毒血凝素(HA)和c-myc标签。在一些情况下，融合结构域具有蛋白酶切割位点(例如因子Xa或凝血酶的切割位点)，其允许相关蛋白酶部分消化融合蛋白，从而从中释放重组蛋白。然后，可通过随后的层析分离，从融合结构域分离出释放的蛋白质。释放的蛋白质然后可通过随后的层析分离从融合结构域中分离。

[0509] 在某些实施方案中，本公开内容的TGF-β超家族I型和/或II型受体多肽含有能够稳定多肽的一个或多个修饰。例如，这类修饰延长多肽的体外半寿期、延长多肽的循环半寿期或降低多肽的蛋白水解性降解。这类稳定修饰包括但不限于融合蛋白(包括例如包含TGF-β超家族I型和/或II型受体多肽结构域和稳定剂结构域的融合蛋白)、糖基化位点的修饰(包括例如糖基化位点加至本公开内容的多肽上)和糖部分的修饰(包括例如从本公开内容的多肽中脱去糖部分)。本文所用术语“稳定剂结构域”不仅仅是指融合蛋白的情况下的融合结构域(例如免疫球蛋白Fc结构域)，而且还包括非蛋白质修饰(例如糖部分)或非蛋白

质部分(例如聚乙二醇)。

[0510] 在优选的实施方案中,按照本文所述方法使用的TGF-β超家族异多聚体复合体是分离的多肽复合体。如本文所用,分离的蛋白质(或蛋白质复合体)或多肽(或多肽复合体)是从其天然环境的组分中分离的蛋白质或多肽。在一些实施方案中,将本公开内容的异多聚体复合体纯化至大于95%、96%、97%、98%或99%纯度,如通过例如电泳(例如SDS-PAGE、等电聚焦(IEF)、毛细管电泳)或层析法(例如离子交换或反相HPLC)测定。用于评价抗体纯度的方法是本领域众所周知的[参见例如Flatman等(2007)J.Chromatogr.B848:79-87]。在一些实施方案中,本公开内容的异多聚体制备物基本不含TGF-β超家族I型受体多肽同多聚体和/或TGF-β超家族II型受体多肽同多聚体。例如在一些实施方案中,异多聚体制备物包含小于约10%、9%、8%、7%、5%、4%、3%、2%或小于1%的TGF-β超家族I型受体多肽同多聚体。在一些实施方案中,异多聚体制备物包含小于约10%、9%、8%、7%、5%、4%、3%、2%或小于1%的TGF-β超家族II型受体多肽同多聚体。在一些实施方案中,异多聚体制备物包含小于约10%、9%、8%、7%、5%、4%、3%、2%或小于1%的TGF-β超家族I型受体多肽同多聚体和小于约10%、9%、8%、7%、5%、4%、3%、2%或小于1%的TGF-β超家族II型受体多肽同多聚体。

[0511] 在某些实施方案中,本公开内容的TGFβ超家族I型和/或II型受体多肽及其异多聚体复合体可通过多种本领域已知技术产生。例如可采用标准蛋白质化学技术例如描述于Bodansky,M.Principles of Peptide Synthesis,Springer Verlag,Berlin(1993)和GrantG.A.(ed.),Synthetic Peptides:A User's Guide,W.H.Freeman and Company,New York(1992)的技术,合成本公开内容的多肽。另外,自动化肽合成仪是市购可获得的(参见例如Advanced ChemTech396型;Milligen/Biosearch9600)。或者,如本领域众所周知的,本公开内容的多肽和复合体,包括其片段或变体,可使用不同的表达系统重组产生[例如大肠杆菌(E.coli)、中华仓鼠卵巢(CHO)细胞、COS细胞、杆状病毒]。在又一个实施方案中,可通过使用例如胰蛋白酶、嗜热菌蛋白酶、胰凝乳蛋白酶、胃蛋白酶或成对碱性氨基酸转化酶(PACE)等蛋白酶,经消化重组产生的全长TGFβ超家族I型和/或II型受体多肽来产生本公开内容的修饰或未修饰的多肽。计算机分析(应用市购可获得的软件,例如MacVector、Omega、PCGene,MolecularSimulation,Inc.)可用来鉴定蛋白水解切割位点。

[0512] 至于结合和拮抗与本公开内容的TGF-βI型受体多肽:TGF-βII型受体多肽异多聚体结合的配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的抗体,考虑抗体可设计为包含结合所述配体表位使得抗体的第一部分与I型受体竞争结合的第一部分和包含结合所述配体的表位使得抗体的第二部分与II型受体竞争结合的第二部分的双特异性抗体。以这种方式,靶向单一配体的双特异性抗体可设计成模拟可通过ALK7:ActRIIB异多聚体赋予的双重I型-II型受体结合阻碍。同样考虑使用两种或更多种抗体的组合可实现相同的作用,其中至少第一抗体结合所述配体的表位,使得第一抗体与I型受体竞争结合,且至少第二抗体结合所述配体的表位,使得第二抗体与II型受体竞争结合。

[0513] B.编码TGFβ超家族I型和/或II型受体多肽的核酸

[0514] 在某些实施方案中,本公开内容提供编码本文公开的TGFβ超家族I型和/或II型受体(包括其片段、功能变体和融合蛋白)的分离和/或重组的核酸。例如SEQ ID NO:12编码天然存在的人ActRIIA前体多肽,而SEQ ID NO:13编码成熟的ActRIIA胞外域。主题核酸可以是单链或双链的。这类核酸可以是DNA或RNA分子。这些核酸可用于例如制备本公开内容的TGF-β超家族异多聚体复合体的方法中。

[0515] 如本文所用,分离的核酸是指从其天然环境的组分中分离的核酸分子。分离的核酸包括通常含有核酸分子的细胞中所含的核酸分子,但核酸分子存在于染色体外或不同于其天然染色体位置的染色体位置。

[0516] 在某些实施方案中,编码本公开内容的TGFβ超家族I型和/或II型受体多肽的核酸要理解为包括是SEQ ID NO:7、8、12、13、16、17、20、21、24、25、28、29、32、33、36、37、40、41、44、45、48、49、52、53、69、70、73、74、77、78、81、82、85、86、89、90、93、94、303、304、307、308、311、312、101、105、113、116、119、122、125、128、131、134、137、140和143中任一个的变体的核酸。变体核苷酸序列包括因一个或多个核苷酸取代、添加或缺失而不同的序列,包括等位基因变体,因此,可包括编码不同于SEQ ID NO:7、8、12、13、16、17、20、21、24、25、28、29、32、33、36、37、40、41、44、45、48、49、52、53、69、70、73、74、77、78、81、82、85、86、89、90、93、94、303、304、307、308、311、312、101、105、113、116、119、122、125、128、131、134、137、140和143中任一个注明的核苷酸序列的序列。

[0517] 在某些实施方案中,本公开内容的TGFβ超家族I型和/或II型受体多肽由与SEQ ID NO:7、8、12、13、16、17、20、21、24、25、28、29、32、33、36、37、40、41、44、45、48、49、52、53、69、70、73、74、77、78、81、82、85、86、89、90、93、94、303、304、307、308、311、312、101、105、113、116、119、122、125、128、131、134、137、140和143有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性的分离或重组的核酸序列编码。本领域普通技术人员应认识到,同与SEQ ID NO:7、8、12、13、16、17、20、21、24、25、28、29、32、33、36、37、40、41、44、45、48、49、52、53、69、70、73、74、77、78、81、82、85、86、89、90、93、94、303、304、307、308、311、312、101、105、113、116、119、122、125、128、131、134、137、140和143互补的序列有至少80%、85%、90%、95%、96%、97%、98%或99%同一性的核酸序列也在本公开内容的范围内。在其它实施方案中,本公开内容的核酸序列可以是分离的、重组的和/或与异源核苷酸序列融合或在DNA文库中。

[0518] 在其它实施方案中,本公开内容的核酸还包括在高严格条件下与SEQ ID NO:7、8、12、13、16、17、20、21、24、25、28、29、32、33、36、37、40、41、44、45、48、49、52、53、69、70、73、74、77、78、81、82、85、86、89、90、93、94、303、304、307、308、311、312、101、105、113、116、119、122、125、128、131、134、137、140和143标明的核苷酸序列、SEQ ID NO:7、8、12、13、16、17、20、21、24、25、28、29、32、33、36、37、40、41、44、45、48、49、52、53、69、70、73、74、77、78、81、82、85、86、89、90、93、94、303、304、307、308、311、312、101、105、113、116、119、122、125、128、131、134、137、140和143的互补序列或其片段杂交的核苷酸序列。本领域普通技术人员应容易理解,可改变促进DNA杂交的合适的严格性条件。例如可在约45℃下在6.0x氯化钠/柠檬酸钠(SSC)中进行杂交,接着在50℃下2.0xSSC洗涤。例如洗涤步骤中的盐浓度可以是选自约2.0xSSC在50℃下的低严格性到约0.2xSSC在50℃下的高严格性。另外,洗涤步骤中的温

度可从在室温约22℃下的低严格性条件提高到约65℃下的高严格性条件。温度和盐两者均可改变,温度或盐浓度任一个保持恒定,同时另一个变量是变化的。在一个实施方案中,本公开内容提供于6xSSC中在室温下的低严格性条件下杂交接着于2xSSC中在室温下洗涤的核酸。

[0519] 因遗传密码中的简并而不同于SEQ ID NO:7、8、12、13、16、17、20、21、24、25、28、29、32、33、36、37、40、41、44、45、48、49、52、53、69、70、73、74、77、78、81、82、85、86、89、90、93、94、303、304、307、308、311、312、101、105、113、116、119、122、125、128、131、134、137、140和143所列核酸的分离的核酸也在本公开内容的范围内。例如多种氨基酸用不止一个三联体指定。指定同一氨基酸的密码子或同义密码子(例如CAU和CAC是组氨酸的同义密码子),可导致不影响蛋白质的氨基酸序列的"沉默"突变。然而,预期在哺乳动物细胞中可存在确实引起主题蛋白质氨基酸序列改变的DNA序列多态性。本领域技术人员认识到,由于天然等位基因变化所致,在给定物种的个体之间,这些变化可存在于编码特定蛋白质的核酸的一个或多个核苷酸(多至约3-5%的核苷酸)之中。任何和所有这类核苷酸变化和所得氨基酸多态性在本公开内容的范围内。

[0520] 在某些实施方案中,在表达构建体中,本公开内容的重组核酸可与一个或多个调节核苷酸序列有效连接。调节核苷酸序列一般可适于用于表达的宿主细胞。本领域已知用于各种宿主细胞的合适表达载体和合适调节序列的许多类型。所述一个或多个调节核苷酸序列通常可包括但不限于启动子序列、前导序列或信号序列、核糖体结合位点、转录起始序列和终止序列、翻译起始序列和终止序列及增强子或激活物序列。本公开内容考虑本领域已知的组成型启动子或诱导型启动子。启动子可以是天然存在的启动子或将不止一种启动子的元件组合的杂合启动子。表达构建体可存在于细胞的附加体(例如质粒)上,或者表达构建体可插入染色体中。在一些实施方案中,表达载体含有选择标记基因以供转化宿主细胞的选择。选择标记基因是本领域众所周知的,并可随所采用的宿主细胞而改变。

[0521] 在本公开内容的某些方面,在包含编码TGFβ超家族I型和/或II型受体多肽并与至少一个调节序列有效连接的核苷酸序列的表达载体中提供主题核酸。调节序列是本领域公认的,并被选择来指导TGFβ超家族I型和/或II型受体多肽的表达。因此,术语调节序列包括启动子、增强子和其它表达调控元件。示例性调节序列描述于Goeddel;Gene Expression Technology:Methods in Enzymology,Academic Press,San Diego,CA(1990)。例如,当与DNA序列有效连接时,控制DNA序列表达的各种表达调控序列的任一种可被用于这些载体以表达编码TGFβ超家族I型和/或II型受体多肽的DNA序列。这类有用的表达调控序列包括例如SV40的早期启动子和晚期启动子、tet启动子、腺病毒或巨细胞病毒立即早期启动子、RSV启动子、lac系统、trp系统、TAC或TRC系统、其表达受T7 RNA聚合酶指导的T7启动子、噬菌体λ的主要操纵基因和启动子区、fd外壳蛋白的调控区、3-磷酸甘油酸激酶或其它糖酵解酶的启动子、酸性磷酸酶启动子(例如Pho5)、酵母α-交配因子启动子、杆状病毒系统的多角体(polyhedron)启动子和已知控制原核细胞或真核细胞或其病毒的基因表达的其它序列及其各种组合。应当理解,表达载体的设计可取决于以下这类因素,例如待转化的宿主细胞的选择和/或欲表达的蛋白质类型。此外,还应考虑载体拷贝数、控制该拷贝数的能力和由该载体编码的任何其它蛋白质(例如抗生素标记)的表达。

[0522] 可通过将克隆的基因或其部分连接至适于在原核细胞、真核细胞(酵母、禽、昆虫

或哺乳动物)或两者中表达的载体中来产生本公开内容的重组核酸。用于产生重组TGFβ超家族I型和/或II型受体多肽的表达载体包括质粒和其它载体。例如,合适的载体包括用于在原核细胞(例如大肠杆菌)中表达的以下类型的质粒:pBR322衍生质粒、pEMBL衍生质粒、pEX衍生质粒、pBTac衍生质粒和pUC衍生质粒。

[0523] 一些哺乳动物表达载体既含有利于载体在细菌中增殖的原核序列,又含有在真核细胞中表达的一个或多个真核转录单位。pcDNAI/amp、pcDNAI/neo、pRc/CMV、pSV2gpt、pSV2neo、pSV2-dhfr、pTk2、pRSVneo、pMSG、pSVT7、pko-neo和pHyg衍生载体是适于真核细胞转染的哺乳动物表达载体的实例。这些载体的一些用来源于细菌质粒(例如pBR322)的序列修饰,以利于在原核细胞和真核细胞两者中复制和进行药物抗性选择。或者,可使用诸如牛乳头瘤病毒(BPV-1)或埃巴病毒(pHEBo、pREP衍生物和p205)等病毒衍生物在真核细胞中瞬时表达蛋白质。其它病毒(包括反转录病毒)表达系统的实例可参见下面基因疗法递送系统的描述。用于质粒制备和宿主生物转化中的各种方法是本领域众所周知的。对于原核和真核细胞两者的其它合适的表达系统以及通用重组方法,参见Molecular Cloning A Laboratory Manual,3rd Ed.,ed.by Sambrook,Fritsch and Maniatis(Cold Spring Harbor Laboratory Press,2001)。在某些情况下,可能需要通过利用杆状病毒表达系统来表达重组多肽。这类杆状病毒表达系统的实例包括pVL衍生载体(例如pVL1392、pVL1393和pVL941)、pAcUW衍生载体(例如pAcUW1)和pBlueBac衍生载体(例如含β-gal的pBlueBac III)。

[0524] 在一个优选的实施方案中,可设计在CHO细胞中产生主题TGFβ超家族I型和/或II型受体多肽的载体,例如Pcmv-Script载体(Stratagene,LaJolla,Calif.)、pcDNA4载体(Invitrogen,Carlsbad,Calif.)和pCI-neo载体(Promega,Madison,Wisc.)。显然,可使用主题基因构建体于在培养基中繁殖的细胞中使主题TGFβ超家族I型和/或II型受体多肽表达,例如产生蛋白质(包括融合蛋白或变体蛋白)以便纯化。

[0525] 本公开内容还涉及用包括主题TGFβ超家族I型和/或II型受体多肽的一种或多种的编码序列的重组基因转染的宿主细胞。宿主细胞可以是任何原核或真核细胞。例如本公开内容的TGFβ超家族I型和/或II型受体多肽可在细菌细胞(例如大肠杆菌)、昆虫细胞(例如利用杆状病毒表达系统)、酵母或哺乳动物细胞[例如中华仓鼠卵巢(CHO)细胞系]中表达。其它合适的宿主细胞为本领域技术人员所知。

[0526] 因此,本公开内容另涉及产生主题TGFβ超家族I型和/或II型受体多肽的方法。例如,可将用编码TGFβ超家族I型和/或II型受体多肽的表达载体转染的宿主细胞在允许TGFβ超家族I型和/或II型受体多肽表达发生的合适条件下培养。可使所述多肽分泌并从细胞和含有所述多肽的培养基的混合物中分离出来。或者,TGFβ超家族I型和/或II型受体多肽可从获自收获并裂解的细胞的胞质或膜部分中分离。细胞培养物包括宿主细胞、培养基和其它副产品。用于细胞培养的合适培养基是本领域众所周知的。可采用本领域已知的用于纯化蛋白质的技术,包括离子交换层析法、凝胶过滤层析法、超滤法、电泳、使用对TGFβ超家族I型和/或II型受体多肽的特定表位有特异性的抗体进行的免疫亲和纯化以及用结合与TGFβ超家族I型和/或II型受体多肽融合的结构域的物质进行的亲和纯化(例如A蛋白柱可用来纯化TGFβ超家族I型受体-Fc和/或II型受体-Fc融合蛋白),将主题多肽从细胞培养基、宿主细胞或两者中分离出来。在一些实施方案中,TGFβ超家族I型和/或II型受体多肽是含有有

利于其纯化的结构域的融合蛋白。

[0527] 在一些实施方案中，纯化通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶(phenylsepharose)层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。TGFβ超家族I型受体-Fc和/或II型受体-Fc融合蛋白可纯化至通过大小排阻层析法测定的>90％、>95％、>96％、>98％或>99％的纯度和通过SDSPAGE测定的>90％、>95％、>96％、>98％或>99％的纯度。目标纯度水平是足以在哺乳动物系统，特别是非人灵长类动物、啮齿动物(小鼠)和人中实现所需结果的水平。

[0528] 在另一个实施方案中，编码纯化前导序列(例如重组TGFβ超家族I型和/或II型受体多肽所需部分的N端上的聚-(His)/肠激酶切割位点序列)的融合基因，可允许使用Ni^{2+}金属树脂通过亲和层析法对表达的融合蛋白进行纯化。然后，纯化前导序列随后可通过用肠激酶处理除去，得到纯化的TGFβ超家族I型和/或II型受体多肽(例如参见Hochuli等(1987)J.Chromatography411:177;以及Janknecht等，PNAS USA88:8972)。

[0529] 制备融合基因的技术是众所周知的。基本上，编码不同多肽序列的各种DNA片段的连接按照常规技术进行，采用用于连接的平端或交错端、限制性内切酶消化以提供合适末端，适当时补平黏性末端，碱性磷酸酶处理以避免不需要的连接，并进行酶促连接。在另一个实施方案中，融合基因可通过常规技术合成，包括自动DNA合成仪。或者，可使用在两个连续基因片段之间产生互补延伸的锚定引物进行基因片段的PCR扩增，所述基因片段随后可退火得到嵌合基因序列(参见例如Current Protocols in Molecular Biology，编辑Ausubel等，John Wiley&Sons:1992)。

[0530] 4.筛选测定法

[0531] 在某些方面，本发明涉及使用TGFβ超家族I型和II型受体异多聚体复合体鉴定作为TGFβ超家族受体的激动剂或拮抗剂的化合物(物质)。可对通过这种筛选法鉴定的化合物进行测试以评价其调节组织例如骨、软骨、肌肉、脂肪和/或神经元的能力；评价其体内或体外调节组织生长的能力。可在例如动物模型中对这些化合物进行测试。

[0532] 有许多针对通过靶向TGFβ超家族配体信号转导(例如SMAD2/3和/或SMAD1/5/8信号转导)调节组织生长来筛选治疗剂的方法。在某些实施方案中，可进行高通量的化合物筛选以鉴定干扰TGFβ超家族受体介导的对所选细胞系的作用的物质。在某些实施方案中，进行所述测定法以筛选和鉴定特异性抑制或降低TGF-β超家族异多聚体复合体与其配偶体，例如TGFβ超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的结合的化合物。或者，所述测定法可用于鉴定增强TGF-β超家族异多聚体复合体与其结合配偶体例如TGFβ超家族配体结合的化合物。在又一个实施方案中，可通过其与本公开内容的TGF-β超家族异多聚体复合体相互作用的能力来鉴定化合物。

[0533] 各种测定形式便可满足需要，然而根据本公开内容，本领域的普通技术人员仍应了解未在本文明确描述的测定形式。如本文所述，可通过任何组合化学方法产生本发明的

试验化合物(作用剂)。或者,主题化合物可以是体内或体外合成的天然存在的生物分子。待测定其起组织生长调节剂作用的能力的化合物(作用剂)可通过例如细菌、酵母、植物或其它生物产生(例如天然产物)、以化学法产生(例如小分子,包括肽模拟物)或以重组法产生。本发明考虑的试验化合物包括非肽基有机分子、肽、多肽、肽模拟物、糖、激素和核酸分子。在一个具体的实施方案中,试验物质是有机小分子,分子量小于约2,000道尔顿。

[0534] 本公开内容的试验化合物可以单一的分散实体提供,或者以较复杂的文库提供,例如通过组合化学制备的文库。这些文库可包含例如醇、烷基卤化物、胺、酰胺、酯、醛、醚和有机化合物的其它类别。向试验系统中提供的试验化合物可呈分离形式或作为化合物的混合物,尤其在最初的筛选步骤中。任选化合物可任选用其它化合物衍生化并具有利于化合物分离的衍生化基团。衍生化基团的非限制实例包括生物素、荧光素、洋地黄毒苷(digoxygenin)、绿色荧光蛋白、同位素、聚组氨酸、磁珠、谷胱甘肽S转移酶(GST)、可光活化的交联剂或其任何组合。

[0535] 在测试化合物和天然提取物文库的许多药物筛选程序中,需要高通量测定法以便使在给定时间内研究的化合物的数目最大化。在无细胞系统(例如可用纯化的或半纯化的蛋白质衍生)中进行的测定法,常常优选作为“初步”筛选,因为可产生无细胞系统以允许快速显现和相对容易地检测由试验化合物介导的分子靶标中的改变。此外,在体外系统中一般可忽略试验化合物的细胞毒性或生物利用度的作用,测定法反而主要集中在药物对分子靶标的作用,如可表现在TGF-β超家族异多聚体复合体与其结合配偶体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)之间结合亲和力的改变。

[0536] 仅举例来说,在本公开内容的示例性筛选测定法中,将目标化合物与通常能够与适用于测定意图的TGF-β超家族配体结合的分离和纯化的TGF-β超家族异多聚体复合体接触。然后向化合物和TGF-β超家族异多聚体复合体的混合物中加入含有合适TGF-β超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的组合物。异多聚体-超家族配体复合体的检测和定量测定提供用于测定化合物抑制(或增强)在TGF-β超家族异多聚体复合体与其结合蛋白之间形成复合体的功效的手段。可通过从使用不同浓度的试验化合物所得到的数据绘制剂量反应曲线,来评价化合物的功效。此外,还可进行对照测定,以提供比较基线。例如,在对照测定中,将分离和纯化的TGF-β超家族配体加入含有TGF-β超家族异多聚体复合体的组合物中,在缺乏试验化合物时,定量测定异多聚体-配体复合体的形成。应了解,一般可以改变可将反应物混合的顺序,并且可以同时混合。此外,可使用细胞提取物和裂解物替换经纯化的蛋白质以提供合适的无细胞测定系统。

[0537] 可通过各种技术,检测TGF-β超家族异多聚体复合体与其它蛋白质的结合。例如,

可使用例如可检测标记的蛋白质，例如放射性标记(例如^{32}P、^{35}S、^{14}C或^{3}H)、荧光标记(例如FITC)或酶标记的TGF-β超家族异多聚体复合体和/或其结合蛋白，通过免疫测定或通过层析检测，定量测定复合体形成的调节。

[0538] 在某些实施方案中，本公开内容考虑在直接或间接测TGF-β超家族异多聚体复合体与其结合蛋白间的相互作用的程度中，采用荧光偏振测定法和荧光共振能量转移(FRET)测定法。此外，其它检测方式，例如基于光波导(参见例如PCT公布号WO96/26432和美国专利号5,677,196)、表面等离振子共振(SPR)、表面电荷传感器和表面力传感器的检测方式都适于本公开内容的许多实施方案。

[0539] 此外，本公开内容考虑采用相互作用陷阱测定法(interaction trap assay)，亦称“双杂合测定法(two hybrid assay)”，鉴定干扰或增强TGF-β超家族异多聚体复合体与其结合配偶体间的相互作用的物质。参见例如美国专利号5,283,317;Zervos等(1993)Cell72:223-232;Madura等(1993)J Biol Chem268:12046-12054;Bartel等(1993)Biotechniques14:920-924;以及Iwabuchi等(1993)Oncogene8:1693-1696)。在一个具体的实施方案中，本公开内容考虑采用反相双杂交系统(reverse two hybrid system)鉴定离解TGF-β超家族异多聚体复合体与其结合蛋白间的相互作用的化合物(例如小分子或肽)[参见例如Vidal和Legrain，(1999)Nucleic Acids Res27:919-29;Vidal和Legrain，(1999)Trends Biotechnol17:374-81;以及美国专利号5,525,490、5,955,280和5,965,368]。

[0540] 在某些实施方案中，通过其与本公开内容的TGF-β超家族异多聚体复合体相互作用的能力来鉴定主题化合物。化合物和TGF-β超家族异多聚体复合体间的相互作用可以是共价的或非共价的。例如，可采用体外生化方法在蛋白质水平上鉴定这类相互作用，包括光致交联、放射性同位素标记配体结合和亲和层析法。参见例如Jakoby WB等(1974)Methods in Enzymology46:1。在某些情况下，可在基于机制的测定法中筛选化合物，例如检测与TGF-β超家族异多聚体复合体结合的化合物的测定法。这可包括固相或液相结合事件。或者，可将编码TGF-β超家族异多聚体复合体的基因与报道系统(例如β-半乳糖苷酶、萤光素酶或绿色荧光蛋白)一起转染至细胞中，优选通过高通量筛选针对文库进行筛选或用文库的各个成员进行筛选。可采用基于其它机制的结合测定法，例如检测自由能变化的结合测定法。结合测定法可用固定于孔、珠粒或芯片或被固定化抗体俘获或被毛细管电泳分离的靶标进行。一般可采用比色终点或荧光或表面等离振子共振检测结合的化合物。

[0541] 5.示例性治疗应用

[0542] 在某些实施方案中，本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可用于治疗或预防与TGFβ超家族受体(例如ALK1、ALK2、ALK3、ALK4、ALK5、ALK6、ALK7、ActRIIA、ActRIIB、BMPRII、TGFBRII和MISRII)和/或TGFβ超家族配体(例如BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、激活素A、激活素B、激活素C、激活素E、激活素AB、激活素AC、激活素AE、激活素BC、激活素BE、nodal、神经胶质细胞衍生神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)的异常活性有关的疾病或病况。这些这样疾病、病症或病况在本文一般称为“TGFβ超家族相关病况”或“TGFβ超家族相关病症”。在某些实施方案中，本发明提供通过给

予个体治疗有效量的本文所述TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合来治疗或预防有需要的个体的方法。本公开内容的任何TGF-β超家族异多聚体复合体可能分别或组合地用于本文公开的治疗应用。这些方法尤其以哺乳动物(包括例如啮齿动物、灵长类动物和人)的治疗性或预防性治疗为目标。

[0543] 本文使用的“预防”病症或病况的治疗药是指以下化合物,其在统计样本中,与未治疗对照样品相比,减少治疗样品中的病症或病况的发生,或者与未治疗对照样品相比,延缓病症或病况的一种或多种症状的发作或者降低病症或病况的一种或多种症状的严重程度。本文使用的术语“治疗”包括指定病况的预防或者病况一旦确立便改善或根除病况。在任一情况下,可通过医师或其它保健护理者提供的诊断和给予治疗剂的预期结果来分辨预防或治疗。

[0544] TGFβ超家族受体-配体复合体在组织生长以及早期发育过程例如不同结构的正确形成或一种或多种发育后能力(包括性发育、垂体激素产生及骨和软骨建立)中起必不可少的作用。因此,TGFβ超家族相关病况/病症包括组织生长异常和发育缺陷。另外,TGFβ超家族相关病况包括但不限于细胞生长和分化的病症,例如炎症、反态反应、自身免疫性疾病、感染性疾病和肿瘤。

[0545] 示例性TGFβ超家族相关病况包括神经肌肉病症(例如肌营养不良和肌肉萎缩)、充血性阻塞性肺病(和与COPD有关的肌肉消耗)、肌肉消耗综合征、肌肉衰减综合征(sarcopenia)、恶病质、脂肪组织病症(例如肥胖症)、2型糖尿病(NIDDM、成人型糖尿病)和骨退变性疾病(例如骨质疏松症)。其它示例性TGFβ超家族相关病况包括肌肉变性病症和神经肌肉病症、组织修复(例如伤口愈合)、神经变性疾病(例如肌萎缩性侧索硬化)和免疫病症(例如与淋巴细胞增殖或功能异常有关的病症)。

[0546] 在某些实施方案中,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合用作治疗肌营养不良的一部分。术语“肌营养不良”是指特征在于骨骼肌和有时心肌和呼吸肌逐步虚弱和衰退的一类退变性肌肉疾病。肌营养不良是特征在于以肌肉的显微镜下变化开始的进行性肌肉消耗和衰弱的遗传病症。在肌肉随时间推移衰退时,人的肌肉强度下降。可用包括主题TGF-β超家族异多聚体复合体的方案治疗的示例性肌营养不良包括:杜兴型肌营养不良(DMD)、贝克肌营养不良(BMD)、Emery-Dreifuss肌营养不良(EDMD)、肢带肌营养不良(LGMD)、面肩胛肱骨肌营养不良(FSH或FSHD)(亦称为Landouzy-Dejerine)、肌强直性营养不良(MMD;亦称为斯坦内特病(Steinert'sDisease))、眼咽肌营养不良(OPMD)、远端肌营养不良(DD)、先天肌营养不良(CMD)。

[0547] 法国神经学家Guillaume Benjamin Amand Duchenne在1860年代最先描述了杜兴型肌营养不良(DMD)。贝克肌营养不良(BMD)以德国医生Peter Emil Becker命名,他在1950年代最先描述了这个DMD变体。DMD是男性中最常见的遗传疾病,3,500名少年中有1人受影响。当位于X染色体短臂上的肌养蛋白基因有缺陷时,便发生DMD。由于男性只携带一拷贝的X染色体,所以他们只有一拷贝的肌养蛋白基因。在没有肌养蛋白蛋白质的情况下,肌肉在收缩和松驰周期内容易受损。虽然在该病早期肌肉被再生补偿,但之后肌肉祖细胞不能跟上持续进行的损害,并且健康肌肉被无功能的纤维脂肪组织代替。

[0548] BMD产生于肌养蛋白基因中的不同突变。BMD患者有部分肌养蛋白,但是或量不足或质量差。部分肌养蛋白的存在保护BMD患者的肌肉免受与DMD患者的肌肉一样严重或快速

地衰退。

[0549] 动物研究表明,抑制GDF8信号转导途径可有效治疗DMD和BMD患者的疾病的不同方面(Bogdanovich等,2002,Nature420:418-421;Pistilli等,2011,Am J Pathol178:1287-1297)。因此,本公开内容的TGF-β超家族异多聚体复合体可用作GDF8抑制剂(拮抗剂),并构成在DMD和BMD患者体内通过GDF8和/或相关的TGFβ超家族配体阻断信号转导的备选方法。

[0550] 同样,本公开内容的TGF-β超家族异多聚体复合体可提供在需要肌肉生长的其它疾病状况下提高肌肉质量的有效方法。例如肌萎缩性侧索硬化(ALS),亦称Lou Gehrig病或运动神经元疾病,是一种侵袭运动神经元(其是引发骨骼肌收缩所必需的中枢神经系统的组分)的慢性、进行性且无法治愈的CNS病症。在ALS中,运动神经元退化并最终死亡,并且尽管人的脑通常保持完整的功能和警觉,但肌肉收缩的引发在脊柱水平上被阻断。发生ALS的个体通常介于40和70岁之间岁,最早退化的运动神经元是支配臂或腿的神经元。患有ALS的患者可能会有行走困难,可能失手掉落东西,摔倒,言语含糊,并且控制不住地笑或哭。当疾病进展时,四肢肌肉因废用而萎缩。肌肉衰弱会使人变得虚弱,患者最终需要轮椅或变得不能下床行动。大多数ALS患者从发病起3-5年内死于呼吸衰竭或死于呼吸机辅助型并发症(如肺炎)。

[0551] 通过TGF-β超家族异多聚体复合体对肌肉质量提高的促进还可有益于患有肌肉消耗疾病的人。Gonzalez-Cadavid等(同上)报道了GDF8表达与人的无脂肪质量负相关,且GDF8基因表达的提高与患有AIDS消耗综合征的男子的体重减轻有关。通过抑制AIDS患者的GDF8功能,可以缓解AIDS的至少某些症状,如果不是完全消除的话,因此显著改善AIDS患者的生命质量。

[0552] 由于在没有营养摄取减少的情况下,GDF8功能的丧失也与脂肪丧失有关(Zimmers等,同上;McPherron和Lee,同上),因此主题TGF-β超家族异多聚体复合体还可用作减慢或预防肥胖症和2型糖尿病发展的治疗药物。

[0553] 癌症厌食-恶病质综合征属于癌症最使人虚弱和危及生命的方面。这种综合征是许多癌症类型中的常见特征—存在于约80%濒临死亡的癌症患者中,不仅仅是造成生命质量差和对化学治疗反应差的原因,而且还是造成与患有相应肿瘤却无体重减轻的患者的生存时间相比生存时间短的原因。如果在6个月时间内发生的非有意体重减轻超过发病前体重的5%,则应怀疑癌症患者有恶病质。与厌食、脂肪和肌肉组织消耗和心理苦恼(psychological distress)有关,恶病质是由癌症和宿主间的复杂相互作用引起的。癌症恶病质影响细胞因子的产生、蛋脂质动员因子和蛋白酶解诱导因子的释放和中间代谢的变化。虽然厌食普遍存在,但仅食物摄取降低不能解释见于癌症患者中的机体组成的改变,而且营养摄取增加不能逆转消耗综合征。目前,没有对控制或逆转恶病质过程的治疗法。由于发现成年小鼠中GDF8的全身性过量表达诱导类似于见于人恶病质综合征中的严重肌肉和脂肪丧失(Zimmers等,同上),因此主题TGF-β超家族异多聚体复合体药物组合物可有益地用于预防、治疗或减轻其中需要肌肉生长的恶病质综合征的症状。可用于预防、治疗或缓解上述肌肉丢失的异聚体复合体的实例是ActRIIB-Fc:ALK4-Fc。

[0554] 在某些实施方案中,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可用于诱导骨和/或软骨形成、防止骨丢失、增加骨矿化、防止骨去矿化和/或增加骨密度的方法。TGF-β超家族异多聚体复合体可用于诊断为亚临床低骨密度的

患者,作为针对骨质疏松症发生的保护手段。

[0555] 在一些实施方案中,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可在人和其它动物的骨折愈合和软骨缺陷中具有医疗用途。主题方法和组合物还可在闭合性和开放性骨折复位术以及改进人工关节固定方面具有预防用途。由成骨剂(osteogenic agent)诱导的从头骨形成可用于先天性、创伤诱发的或肿瘤切除术引起的颅面缺陷的修复,并且也可用于美容整形手术。此外,本发明的方法和组合物可用于牙周病和其它牙修复过程的治疗。在某些情况下,TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可提供吸引成骨细胞、刺激成骨细胞生长或诱导成骨细胞祖细胞分化的环境。本公开内容的TGF-β超家族异多聚体复合体还可用于治疗骨质疏松症。此外,TGF-β超家族异多聚体复合体可用于软骨缺陷的修复和骨关节炎的预防/逆转。如上所述可用于诱导骨形成、防止骨丢失、增加骨矿化、防止骨去矿化和/或增加骨密度的异聚体复合体的实例是ActRIIB-Fc:ALK3-Fc和ActRIIB-Fc:ALK4-Fc。

[0556] Rosen等(编辑)Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,7thed.American Society for Bone and Mineral Research,Washington D.C.(通过引用结合到本文)提供可从属于用TGF-β超家族异多聚体复合体或与TGF-β超家族异多聚体复合体的组合治疗的骨病的详尽论述。本文提供部分名单。本发明的方法和组合物可适用于特征是骨丢失或引起骨丢失的病况,例如骨质疏松症(包括继发性骨质疏松症)、甲状旁腺功能亢进、慢性肾病-矿物质和骨异常(chronic kidney disease mineral bone disorder)、性激素剥夺或切除(例如雄激素和/或雌激素)、糖皮质激素治疗、类风湿性关节炎、重度烧伤、甲状旁腺功能亢进、高钙血症、低钙血症、低磷酸盐血症、骨软化(包括瘤源性软骨病)、高磷酸盐血症、维生素D缺乏症、甲状旁腺功能亢进(包括家族性甲状旁腺功能亢进)和假性甲状旁腺功能减退症、肿瘤骨转移、肿瘤或化学疗法引起的骨丢失、骨与骨髓肿瘤(例如多发性骨髓瘤)、缺血性骨病、牙周病和口腔骨丢失、库欣病(Cushing's disease)、佩吉特病(Paget's disease)、甲状腺毒症、慢性腹泻状态或吸收不良、肾小管性酸中毒或神经性厌食症。本发明的方法和组合物还可用于以骨形成或愈合失败为特征的病况,包括不愈合骨折、在其它方面愈合缓慢的骨折、胎儿和新生儿骨发育异常(例如低钙血症、高钙血症、钙受体缺陷和维生素D缺乏症)、骨坏死(包括颌骨坏死)和成骨不全。另外,合成代谢作用可引起这类拮抗剂减轻与骨损伤或侵蚀相关的骨疼痛。由于抗吸收作用,这类拮抗剂可用于治疗异常骨形成的病症,例如成骨细胞肿瘤转移(例如原发性前列腺癌或乳腺癌有关的肿瘤转移)、骨原性骨肉瘤、骨硬化症、进行性骨干发育异常、骨内膜骨质增生(endosteal hyperostosis)、全身脆弱性骨硬化和肢骨纹状肥大。可治疗的其它病症包括纤维性结构不良和软骨发育不全。

[0557] 在另一个具体的实施方案中,本公开内容提供用于修复骨折和与软骨和/或骨缺陷或牙周病有关的其它病况的治疗方法和组合物。本发明进一步提供用于伤口愈合和组织修复的治疗方法和组合物。伤口的类型包括但不限于烧伤、切口和溃疡。参见例如PCT公布号WO84/01106。这类组合物包含与药物可接受的溶媒、载体或基质混合的治疗有效量的本公开内容的TGF-β超家族异多聚体复合体的至少一种。

[0558] 在一些实施方案中,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可适用于引起骨丢失的病况,例如骨质疏松症、甲状旁腺功能亢进、库

欣病、甲状腺毒症、慢性腹泻状态或吸收不良、肾小管性酸中毒或神经性厌食症。通常认为，作为女性、具有低体重和有久坐生活方式是骨质疏松症的风险因子(骨矿物质密度丢失，导致骨折风险)。然而，骨质疏松症还可产生于某些药物的长期使用。产生于药物或另一种医学病况的骨质疏松症称为继发性骨质疏松症。在库欣病中，由机体产生的过量皮质醇引起骨质疏松症和骨折。与继发性骨质疏松症有关的最普通的药物是皮质甾类，一种像由肾上腺天然产生的激素皮质醇一样起作用的药物类别。虽然骨骼发育需要适当水平的甲状腺激素，但过量的甲状腺激素可随时间降低骨质量。含有铝的抗酸药当被有肾问题的人、特别进行透析的人以高剂量服用时，可引起骨丢失。可引起继发性骨质疏松症的其它药物包括用于预防癫痫发作的苯妥英(Dilantin)和巴比妥类;甲氨蝶呤(Rheumatrex，Immunex，Folex PFS)，一种用于某些形式的关节炎、癌症和免疫疾病的药物;环孢菌素(Sandimmune，Neoral)，一种用于治疗某些自身免疫病和抑制器官移植患者的免疫系统的药物;促黄体激素释放激素激动剂(Lupron、Zoladex)，用于治疗前列腺癌和子宫内膜异位症;肝素(Calciparine、Liquaemin)，一种抗凝药;以及消胆胺(Questran)和考来替泊(Colestid)，用于治疗高胆固醇。产生于癌症疗法的骨丢失是普遍公认的，并称为癌症疗法诱导的骨丢失(CTIBL)。骨转移可在骨中产生空腔，这可通过用TGF-β超家族异多聚体复合体治疗来纠正。骨丢失还可能由牙龈病(一种其中位于牙龈隐窝的细菌产生毒素和有害酶的慢性感染)引起。

[0559] 在又一个实施方案中，本公开内容提供用于治疗与异常或不需要的骨生长有关的疾病或病症的方法和治疗剂。例如患有先天病症进行性骨化性纤维发育不良(FOP)的患者受累于自发或在响应组织创伤时软组织中的进行性异位骨生长，其对生命质量造成重大影响。另外，异常骨生长可发生在髋置换手术之后，因此破坏手术结果。这是一种更常见的病理性骨生长的实例，且其中主题方法和组合物的情况可能是治疗有益的。相同的方法和组合物也可用于治疗其它形式的异常骨生长(例如在创伤、烧伤或脊髓损伤后骨的病理性骨生长)，并且用于治疗或预防与见于与转移性前列腺癌或骨肉瘤关联的异常骨生长有关的不良病况。

[0560] 在某些实施方案中，本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可用于促进癌症患者的骨形成。患有某些肿瘤(例如前列腺肿瘤、乳腺肿瘤、多发性骨髓瘤或引起甲状旁腺功能亢进的任何肿瘤)的患者因肿瘤诱导的骨丢失、骨转移和治疗剂可能有骨丢失的高风险。甚至在缺少骨丢失或骨转移的证据时，这类患者也可用TGF-β超家族异多聚体复合体或复合体的组合治疗。还可监测患者的骨丢失或骨转移的证据，且在指示物表明风险增加的情况下，可用TGF-β超家族异多聚体复合体治疗患者。一般而言，采用DEXA扫描评价骨密度的变化，同时骨重建的指示物可用于评价骨转移的可能性。可监测血清标志物。骨特异性碱性磷酸酶(BSAP)是一种存在于成骨细胞中的酶。患有骨转移和导致骨重建增加的其它病况的患者中，BSAP的血液水平升高。骨钙蛋白和前胶原肽也与骨形成和骨转移有关。在患有由前列腺癌引起的骨转移的患者中检出BSAP增加，在乳腺癌所致骨转移中增加的程度较低。在已转移至骨的前列腺癌中，BMP7水平高，但在由膀胱癌、皮肤癌、肝癌或肺癌所致骨转移中，BMP7不高。I型羧基端端肽(ICTP)是存在于骨吸收期间形成的胶原中的交联物。因为骨不断破坏和重建，ICTP可存在于整个机体中。然而，在骨转移部位，水平可显著高于正常骨的区域。在由前列腺癌、肺癌和乳腺癌所致骨转移中存

在高水平的ICTP。另一种胶原交联物，I型N端端肽(NTx)在骨更新期间随ICTP一起产生。在由许多不同类型的癌症(包括肺癌、前列腺癌和乳腺癌)引起的骨转移中，NTx的量增加。同样，NTx的水平随骨转移进程提高。因此，这种标志物既可用于检测转移，又可测定疾病的程度。吸收的其它标志物包括吡啶诺林(pyridinoline)和脱氧吡啶诺林。吸收标志物或骨转移标志物的任何增加都表明患者需要TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合治疗。

[0561] 本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可与其它骨活性药物一同给予。可通过给予单一共制剂、同时给予或在不同时间给予实现共同给药。TGF-β超家族异多聚体复合体如果与其它骨活性剂一起给予，可能是特别有利的。患者可获益于同时接受TGF-β超家族异多聚体复合体和服用钙补充剂、维生素D、适当运动和/或在一些情况下的其它药物。其它药物的实例包括二膦酸盐(阿仑膦酸盐、伊班膦酸盐和利塞膦酸盐)、降钙素、雌激素、甲状旁腺激素和雷洛昔芬。二膦酸盐(阿仑膦酸盐、伊班膦酸盐和利塞膦酸盐)、降钙素、雌激素和雷洛昔芬影响骨重建周期，被归类为抗吸收药物。骨重建包括两个截然不同的阶段：骨吸收和骨形成。抗吸收药物减慢或终止骨重建周期的骨吸收部分，但不减慢该周期的骨形成部分。因此，以比骨吸收大的速度持续新的形成，而且骨密度可随时间增加。特立帕肽，一种甲状旁腺激素形式，在骨重建周期中提高骨形成的速度。阿仑膦酸盐获准用于预防(5mg/天或一周一次35mg)和治疗(10mg/天或一周一次70mg)绝经后骨质疏松症。阿仑膦酸盐减少骨丢失，增加骨密度和降低脊柱、腕和髋骨折的风险。阿仑膦酸盐还获准用于治疗由于长期使用这些药物(即泼尼松和可的松)所致男性和女性的糖皮质激素诱发性骨质疏松症并用于治疗男性骨质疏松症。阿仑膦酸盐加维生素D获准用于治疗绝经后女性的骨质疏松症(70mg一周一次加维生素D)，以及用于治疗以改善患有骨质疏松症的男性的骨质量。伊班膦酸盐获准用于预防和治疗绝经后骨质疏松症。一月一次以丸剂服用(150mg)，伊班膦酸盐应在每个月的同一天服用。伊班膦酸盐减少骨丢失、增加骨密度和降低脊柱骨折风险。利塞膦酸盐获准用于预防和治疗绝经后骨质疏松症。每日(5mg剂量)或每周(35mg剂量或35mg剂量与钙)服用，利塞膦酸盐减缓骨丢失，增加骨密度并降低脊柱和非脊柱骨折的风险。利塞膦酸盐还获准用于男性和女性以预防和/或治疗由于长期使用这些药物(即泼尼松或可的松)引起的糖皮质激素诱发性骨质疏松症。降钙素是天然存在的参与钙调节和骨代谢的激素。在绝经期后5年以上的女性中，降钙素减缓骨丢失、增加脊柱骨密度，并可缓解骨折相关疼痛。降钙素降低脊柱骨折的风险。可以注射剂(每日50-100IU)或鼻腔喷雾剂(每日200IU)获得降钙素。

[0562] 患者还可获益于共同接受TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合和其它的骨活性药物。雌激素疗法(ET)/激素疗法(HT)获准用于预防骨质疏松症。已表明ET减少骨丢失，增加脊柱和髋两者的骨密度，并降低绝经后女性的髋和脊柱骨折的风险。最常以递送约每日0.3mg的低剂量或约每日0.625mg的标准剂量的丸剂或皮肤贴剂形式给予ET，甚至在70岁以后开始给予时也有效。当只服用雌激素时，可能增加女性发生子宫膜癌(子宫内膜癌)的风险。为了排除这种风险，医疗保健人员将激素孕酮与雌激素(激素替代疗法或HT)联合开给有完整子宫的女性。ET/HT缓解绝经期症状，并显示对骨健康具有有益作用。副作用可包括阴道出血、乳房触痛、情绪障碍和胆囊疾病。雷洛昔芬，一天60mg，获准用于预防和治疗绝经后骨质疏松症。它来自一类已开发以提供雌激素的有益作

用而无其潜在缺点的称为选择性雌激素受体调节剂(SERM)的药物类别。雷洛昔芬提高骨质量,并降低脊柱骨折风险。尚未获得表明雷洛昔芬可降低髋骨折和其它非脊柱骨折的风险的数据。特立帕肽,一种甲状旁腺激素形式,获准用于治疗处于高骨折风险的绝经后女性和男性的骨质疏松症。这种药物刺激新骨形成,并显著增加骨矿物质密度。在绝经后女性中,在脊柱、髋、足、肋和腕中注意到骨折减少。在男性中,在脊柱中注意到骨折减少,但没有足够的数据评价其它部位的骨折减少。特立帕肽以每日注射剂自我给药长达24个月。

[0563] 在其它实施方案中,TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可用于调节调节动物的体脂肪含量和用于治疗或预防其相关病况、特别是其损害健康的相关病况的组合物和方法。按照本发明,调节(控制)体重可指减轻或增加体重、降低或提高体重增加的速度或者提高或降低体重减轻的速度,并且还包括主动保持或不显著改变体重(例如针对可能以其它方式增加或降低体重的外部或内部影响)。本公开内容的一个实施方案涉及通过将本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合给予有需要的动物(例如人)来调节体重。

[0564] 在一些实施方案中,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可用于在动物中减轻体重和/或降低体重增加,更特别是用于治疗或改善有肥胖症风险或患有肥胖症的患者的肥胖症。在另一个具体实施方案中,本发明涉及用于治疗不能增加体重或保持体重的动物(例如患有消耗综合征的动物)的方法和化合物。这类方法有效增加体重和/或身体质量,或减轻体重和/或减轻质量丧失,或改善与/由不良性的低(例如不健康)体重和/或身体质量有关/引起的病况。另外,高胆固醇病症(例如高胆固醇血症或脂血症障碍(dislipidemia))可用本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合治疗。

[0565] 在某些方面,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可用来在有需要的受试者中提高红细胞水平、治疗或预防贫血和/或治疗或预防无效红细胞生成。在某些方面,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可与用于提高红细胞水平的常规治疗方法,特别是用于治疗多因素起因贫血的常规治疗方法联用。用于提高红细胞水平的常规治疗方法包括例如红细胞输注、给予一种或多种EPO受体激活物、造血干细胞移植、免疫抑制生物制剂和药物(例如皮质甾类)。在某些实施方案中,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可用于在有需要的受试者中治疗或预防无效红细胞生成和/或与无效红细胞生成有关的病症。在某些方面,本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合可与用于治疗或预防贫血或无效红细胞生成障碍的常规治疗方法,特别是用于治疗多因素起因贫血的常规治疗方法联用。

[0566] 一般而言,本公开内容所述疾病或病况的治疗或预防通过以“有效量”给予本公开内容的TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合来实现。药剂的有效量是指以达到所需治疗或预防结果所必需的剂量并持续一段时间的有效的量。本公开内容的药剂的“治疗有效量”可随例如个体的疾病状态、年龄、性别和体重及作用剂在个体中引起所需反应的能力等因素而变化。“预防有效量”是指以达到所需预防结果所必需的剂量并持续一段时间的有效的量。

[0567] 在某些实施方案中,TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体

的组合，任选与EPO受体激活物混合，可用于增加健康个体和所选患者群的红细胞、血红蛋白或网织红细胞水平。合适的患者群的实例包括具有不良性低红细胞或血红蛋白水平的患者(例如贫血患者)和有发育中的不良性低红细胞或血红蛋白水平的风险的患者，例如将要进行大手术或可引起大量失血的其它手术的那些患者。在一个实施方案中，有足够红细胞水平的患者用TGF-β超家族异多聚体复合体或TGF-β超家族异多聚体复合体的组合治疗以提高红细胞水平，然后抽出血液，保存待稍后输血用。

[0568] 任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体，可在贫血患者中用于提高红细胞水平、血红蛋白水平和/或血细胞比容水平。在观察人的血红蛋白和/或血细胞比容水平时，对于适当年龄和性别类别小于正常的水平可表示有贫血，但要考虑个体变化。例如10-12.5g/dl的血红蛋白水平，通常约11.0g/dl被视为在健康成年人的正常范围内，然而，就疗法而言，较低的目标水平可引起较少的心血管副作用[参见例如Jacobs等(2000)Nephrol Dial Transplant15,15-19]。或者，血细胞比容水平(由细胞占据的血样体积的百分比)可用作贫血的度量。健康个体的血细胞比容水平对于成年男性为约41-51％，对于成年女性为35-45％。在某些实施方案中，患者可用欲使患者恢复到红细胞、血红蛋白和/或血细胞比容的目标水平的给药方案治疗。由于血红蛋白和血细胞比容水平在人与人之间不同，最好针对每个患者，分别考虑目标血红蛋白和/或血细胞比容水平。

[0569] 在患有组织损伤、感染和/或慢性疾病，特别是癌症的患者中常常观察到贫血。在一些受试者中，贫血以低红细胞生成素水平和/或对骨髓中的红细胞生成素的不当反应为特征[参见例如Adamson(2008)Harrison's Principles of Internal Medicine,17th ed.;McGraw Hill,New York,pp628-634]。贫血的可能病因包括例如失血、缺乏营养(例如蛋白质膳食摄入减少)、药物反应、与骨髓有关的各种问题和多种疾病。更具体地说，贫血与包括例如以下的多种病症和病况有关：骨髓移植；实体瘤(例如乳腺癌、肺癌和结肠癌)；淋巴系统肿瘤(例如慢性淋巴细胞白血病、非霍奇金淋巴瘤和霍奇金淋巴瘤)；造血系统肿瘤(例如白血病、骨髓增生异常综合征和多发性骨髓瘤)；放射疗法；化学疗法(例如含铂的方案)；炎性疾病和自身免疫性疾病，包括但不限于类风湿性关节炎、其它炎性关节炎、系统性红斑狼疮(SLE)、急性或慢性皮肤疾病(例如银屑病)、炎性肠病(例如克罗恩病和溃疡性结肠炎)；急性或慢性肾病或肾衰竭，包括特性性或先天性病况；急性或慢性肝病；急性或慢性出血；其中因患者同种抗体或自身抗体和/或出于宗教原因(例如一些耶和华见证人)不可能输入红细胞的情况；感染(例如疟疾和骨髓炎)；血红蛋白病，包括例如镰状细胞病(贫血)、珠蛋白生成障碍性贫血；药物使用或滥用(例如酒精滥用)；因任何原因避免输血的患有贫血的儿科患者；和年长患者或患有基础性心肺疾病伴贫血、因担心有关循环超负荷而不能接受输血的患者[参见例如Adamson(2008)Harrison's Principles of Internal Medicine,17th ed.;McGraw Hill,New York,pp628-634]。在一些实施方案中，本公开内容的一种或多种TGF-β超家族异多聚体复合体可用来治疗或预防与本文公开的一种或多种病症或病况有关的贫血。

[0570] 许多因素可对癌症相关贫血产生影响。一些与疾病过程本身和炎性细胞因子(例如白介素-1、干扰素-γ)和肿瘤坏死因子的产生有关[Bron等(2001)Semin Oncol 28(Suppl8):1-6]。在它的作用当中，炎症诱导关键的铁-调节肽hepcidin，从而抑制铁自巨噬

细胞输出，常常限制用于红细胞生成的铁可用性[参见例如Ganz(2007)J Am Soc Nephrol18:394-400]。不同途径失血也可引起癌症相关贫血。因癌症进展所致贫血的发生率随癌症类型而变化，血细胞比容水平为前列腺癌中的5％到多发性骨髓瘤高达90％。癌症相关贫血对患者有重大后果，包括疲劳和生命质量下降、治疗功效降低和死亡率升高。在一些实施方案中，任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体，可用来治疗癌症相关贫血。

[0571] 造血障碍性贫血可产生于骨髓的原发性功能障碍或衰竭。造血障碍性贫血包括：慢性病贫血、肾病贫血、代谢减退状态相关贫血和癌症相关贫血。在这些类型的每种中，内源性红细胞生成素水平对于所观察到的贫血程度不适当地低。其它造血障碍性贫血包括：早期缺铁性贫血和因骨髓损害引起的贫血。在这些类型中，内源性红细胞生成素水平对于所观察到的贫血程度不适当地升高。突出的实例可为由癌症和/或化学治疗药物或癌症放射疗法引起的骨髓抑制。临床试验的广泛综述发现轻度贫血可在化学疗法后发生于100％的患者中，而较严重的贫血可发生在高达80％的这类患者中[参见例如Groopman等(1999)J Natl Cancer Inst91:1616-1634]。骨髓抑制药物包括例如：1)烷化剂例如氮芥(例如美法仑)和亚硝基脲(例如链佐星)；2)抗代谢药例如叶酸拮抗剂(例如甲氨蝶呤)、嘌呤类似物(例如硫鸟嘌呤)和嘧啶类似物(例如吉西他滨)；3)细胞毒性抗生素例如蒽环类(例如多柔比星)；4)激酶抑制剂(例如吉非替尼)；5)有丝分裂抑制剂例如紫杉烷类(例如紫杉醇)和长春花属生物碱(例如长春瑞滨)；6)单克隆抗体(例如利妥昔单抗)；和7)拓扑异构酶抑制剂(例如托泊替康和依托泊苷)。另外，导致低代谢率的病况可产生轻度-中度造血障碍性贫血。在这类病况中有内分泌缺乏状态。例如贫血可发生在艾迪生病(Addison'sdisease)、甲状腺机能减退、甲状旁腺功能亢进或去雄或用雌激素治疗的男性。在一些实施方案中，任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体，可用来治疗造血障碍性贫血。

[0572] 慢性肾病有时与造血障碍性贫血有关，且贫血程度在严重程度上随肾损害水平变化。这类贫血主要由红细胞生成素产生不足和红细胞存活减少引起。慢性肾病通常在数年或数十年间逐步进行到末期(第5期)疾病，在该点上患者生存需要透析或肾移植。贫血常常在该进程早期发生，并随疾病进展恶化。肾病所致贫血的临床结果被充分证实，包括发生左心室肥大、认知功能减退、生命质量下降和免疫功能改变[参见例如Levin等(1999)Am J KidneyDis27:347-354;Nissenson(1992)Am J Kidney Dis20(Suppl1):21-24;Revicki等(1995)Am J Kidney Dis25:548-554;Gafter等(1994)Kidney Int45:224-231]。在一些实施方案中，任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体，可用来治疗与急性或慢性肾病或肾衰竭有关的贫血。

[0573] 产生于足量体积的急性失血(例如来自创伤或产后出血)的贫血称为急性出血后贫血。急性失血最初引起血容量减少而无贫血，因为存在RBC连同血液组分成比例的耗减。然而，血容量减少可快速引发将流体自血管外转移到血管区室的生理机制，这导致血液稀释和贫血。如果是慢性的话，失血逐步耗减体内铁储存并最终导致铁缺乏。在一些实施方案中，任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体，可用来治疗由急性失血引起的贫血。

[0574] 缺铁性贫血在铁缺乏增加的逐步进展中是终末期，所述铁缺乏包括作为中期的铁

负平衡和缺铁性红细胞生成。铁缺乏可产生于铁需求增加、铁摄取减少或铁丢失增加，正如以例如妊娠、不当饮食、肠吸收不良、急性或慢性炎症和急性或慢性失血等病况为例说明的。在该类型的轻度-中度贫血的情况下，骨髓保持低增殖，且RBC形态大多正常；然而，即使轻度贫血也可产生一些小细胞低色素性RBC，且向重度铁-缺陷型贫血的转变伴有骨髓的过度增殖和日益普遍的小红细胞和血红蛋白过少的RBC[参见例如Adamson(2008)Harrison's Principles of Internal Medicine,17th ed.;McGraw Hill,New York,pp 628-634]。缺铁性贫血的合适疗法取决于其病因和严重程度，以口服铁制剂、胃肠外铁制剂和RBC输入作为主要常规选择。在一些实施方案中，任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体，可用来治疗慢性铁缺乏。

[0575] 骨髓增生异常综合征(MDS)是一类多种多样的血液病况，其特征在于骨髓血细胞产生失效和转化成急性髓细胞性白血病的风险。在MDS患者中，血液干细胞不长成健康的红细胞、白细胞或血小板。MDS病症包括例如难治性贫血、带有环铁粒幼红细胞的难治性贫血、带有过量胚细胞的难治性贫血、带有转化中的过量胚细胞的难治性贫血、难治性血细胞减少症伴多谱系发育异常和与分离的5q染色体异常相关的骨髓增生异常综合征。因为这些病症在造血细胞的数量和质量两方面出现不可逆的缺陷，所以大多数MDS患者患上慢性贫血。因此，MDS患者最终需要血液输血和/或用生长因子(例如红细胞生成素或G-CSF)治疗以提高红细胞水平。然而，许多MDS患者出现因这类疗法的频率引起的副作用。例如经常性频繁红细胞输注的患者显示因额外铁积累引起组织和器官损害。因此，本公开内容的一种或多种TGF-β超家族异多聚体复合体，可用于治疗患有MDS的患者。在某些实施方案中，患有MDS的患者可使用任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗。在其它实施方案中，患有MDS的患者可使用本公开内容的一种或多种TGF-β超家族异多聚体复合体和一种或多种用于治疗MDS的其它治疗剂的组合治疗，所述其它治疗剂包括例如沙利度胺、来那度胺、阿扎胞苷、地西他滨、红细胞生成素、去铁胺、抗胸腺细胞球蛋白和非格司亭(G-CSF)。

[0576] 根据铁动力学研究最初与再生障碍性贫血、出血或外周性溶血区分开来[参见例如Ricketts等(1978)Clin Nucl Med3:159-164]，无效红细胞生成描述了鉴于存在于骨髓中的祖红细胞(成红细胞)的数目其中成熟RBC的产生小于可预期的数目的各种各样的贫血[Tanno等(2010)Adv Hematol 2010:358283]。在这类贫血中，组织缺氧持续，尽管红细胞生成素水平因无效的成熟RBC产生所致升高。有缺陷的循环最终发展成其中升高的红细胞生成素水平驱动成红细胞大量扩增，甚至在治疗性RBC输入下，都可能因髓外红细胞生成[参见例如Aizawa等(2003)Am J Hematol74:68-72]、成红细胞诱导的骨病理[参见例如DiMatteo等(2008)J Biol Regul Homeost Agents22:211-216]和组织铁超负荷所致导致脾大(脾增大)[参见例如Pippard等(1979)Lancet2:819-821]。因此，通过提高红细胞生成有效性，本公开内容的一种或多种TGF-β超家族异多聚体复合体可打破前述循环，因此不仅仅减轻基础性贫血，而且还减轻红细胞生成素水平升高、脾大、骨病理和组织铁超负荷的相关并发症。在一些实施方案中，本公开内容的一种或多种TGF-β超家族异多聚体复合体可用于治疗或预防无效红细胞生成，包括贫血和EPO水平升高以及并发症例如脾大、成红细胞诱导的骨病理、铁超负荷及其伴之而来的病理。至于脾大，这类病理包括胸痛或腹痛和网状内皮增生。髓外血细胞生成不仅可发生在脾中，还可能以髓外造血性假性肿瘤的形式存在于

其它组织中[参见例如Musallam等(2012)Cold Spring Harb Perspect Med 2:a013482]。至于成红细胞诱导的骨病理,伴之而来的病理包括低骨矿物质密度、骨质疏松症和骨痛[参见例如Haidar等(2011)Bone 48:425-432]。至于铁超负荷,伴之而来的病理包括hepcidin抑制和膳食铁吸收过多[参见例如Musallam等(2012)Blood Rev26(Suppl1):S16-S19]、多发性内分泌病和肝纤维变性/肝硬化[参见例如Galanello等(2010)Orphanet J Rare Dis 5:11]和铁超负荷心肌病[Lekawanvijit等,2009,Can J Cardiol 25:213-218]。

[0577] 无效红细胞生成的最常见的原因是珠蛋白生成障碍性贫血综合征、其中完整α-和β-血红蛋白链产生失衡导致成红细胞成熟期间细胞凋亡增加的遗传性血红蛋白病[参见例如Schrier(2002)Curr Opin Hematol 9:123-126]。珠蛋白生成障碍性贫血总体上是全世界最常见的遗传病症,预测在美国和全球其变动的流行病学模式引起不断增长的公共健康问题[Vichinsky(2005)Ann NY Acad Sci 1054:18-24]。珠蛋白生成障碍性贫血综合征根据其严重程度命名。因此,α-珠蛋白生成障碍性贫血包括轻型α-珠蛋白生成障碍性贫血(亦称为α-珠蛋白生成障碍性贫血特性;2个受影响的α-珠蛋白基因)、血红蛋白H病(3个受影响的α-珠蛋白基因)和重型α-珠蛋白生成障碍性贫血(亦称为卵泡积液;4个受影响的α-珠蛋白基因)。β-珠蛋白生成障碍性贫血包括轻型β-珠蛋白生成障碍性贫血(亦称为β-珠蛋白生成障碍性贫血特性;1个受影响β-珠蛋白基因)、中间型β-珠蛋白生成障碍性贫血(2个受影响的β-珠蛋白基因)、血红蛋白E珠蛋白生成障碍性贫血(2个受影响的β-珠蛋白基因)和重型β-珠蛋白生成障碍性贫血(亦称为库利氏贫血(Cooley's anemia);2个受影响的β-珠蛋白基因,造成β-珠蛋白蛋白质完全缺乏)。β-珠蛋白生成障碍性贫血影响多个器官,与可观的发病率和死亡率有关,并且目前需要终身治疗。虽然患有β-珠蛋白生成障碍性贫血的患者预期寿命近年来因使用与铁螯合疗法组合的常规输血所致延长,但产生于输血和铁的过量胃肠吸收的铁超负荷可引起严重的并发症,例如心脏病、血栓形成、性腺功能减退症、甲状腺机能减退、糖尿病、骨质疏松症和骨质减少[参见例如Rund等(2005)N Engl J Med 353:1135-1146]。在某些实施方案中,任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体,可用于治疗或预防珠蛋白生成障碍性贫血综合征。

[0578] 在一些实施方案中,除珠蛋白生成障碍性贫血综合征以外,任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体还可用于治疗无效红细胞生成的病症。这类病症包括铁粒幼细胞贫血(遗传性或先天性);红细胞生成异常性贫血(I和II型);镰状细胞性贫血;遗传性球形细胞增多症;丙酮酸激酶缺乏;可能由例如叶酸缺乏(因先天疾病、摄取减少或需求增加所致)、钴胺素缺乏(因先天疾病、恶性贫血、吸收减低、胰功能不全或摄取减少所致)、某些药物或不明原因(先天性红细胞生成异常性贫血、难治性巨红细胞性贫血或红白血病)等情况引起的巨红细胞性贫血;包括例如骨髓纤维变性(骨髓组织异生)和脊髓痨在内的骨髓病性贫血;先天性红细胞生成性卟啉症和铅中毒。

[0579] 在某些实施方案中,本公开内容的一种或多种TGF-β超家族异多聚体复合体可与用于无效红细胞生成的支持疗法联用。这类疗法包括输入红细胞或全血以治疗贫血。在慢性或遗传性贫血中,铁稳态的正常机制被重复输血克制,最终导致铁在例如心脏、肝和内分泌腺等活组织中的中毒性和可能致死蓄积。因此,对患有慢性无效红细胞生成的患者的支持疗法还包括用一种或多种铁螯合分子处理以促进尿和/或大便中的铁排泄,从而防止或逆转组织铁超负荷[参见例如Hershko(2006)Haematologica91:1307-1312;Cao等(2011),

Pediatr Rep3(2):e17]。有效的铁螯合剂应能够选择性结合和中和三价铁这种非运铁蛋白结合铁的氧化形式,这可能解释大多数的铁毒性通过催化产生羟基和氧化产物[参见例如Esposito等(2003)Blood 102:2670-2677]。这些螯合剂在结构上是多样化的,但所有都具有氧或氮供体原子能够与单个铁原子以1:1(六齿螯合剂)、2:1(三齿)或3:1(二齿)的化学计量关系形成中和性八面体配位络合物[Kalinowski等(2005)Pharmacol Rev 57:547-583]。一般而言,有效的铁螯合剂还是相对低分子量的(例如小于700道尔顿),在水和脂质两者中的溶解度使得能够接近受累组织。铁螯合分子的具体实例包括去铁胺、需要每日胃肠外给予的细菌源的六齿螯合剂和口服活性合成剂去铁酮(二齿)和地拉罗司(三齿)。由同日给予两种铁螯合剂组成的组合疗法在对螯合疗法单一疗法无反应的患者中显示出希望,并且还克服了仅用去铁胺顺应性差的患者中的问题[Cao等(2011)Pediatr Rep3(2):e17;Galanello等(2010)Ann NY Acad Sci1202:79-86]。

[0580] 如本文所用,“与……组合”或“共同给药”是指使得第二疗法在体内仍然有效的任何给药形式(例如两种化合物在患者中同时有效,这可包括两种化合物的协同效应)。有效性可能与药剂在血液、血清或血浆中的可测量浓度无关。例如不同的治疗药化合物可在同一制剂或单独的制剂中共同或序贯给予并以不同的方案给予。因此,接受这类治疗的个体可获益于不同疗法的综合作用。本公开内容的一种或多种TGF-β超家族异多聚体复合体可与一种或多种其它另外的药剂或支持疗法同时、在其之前或之后给予。一般而言,可以针对该具体治疗剂确定的某一剂量和/或按某一时间表给予每种治疗剂。在方案中采用的具体组合应考虑本公开内容的拮抗剂与疗法和/或要达到的所需治疗作用的相容性。

[0581] 在某些实施方案中,对于无效红细胞生成,本公开内容的一种或多种TGF-β超家族异多聚体复合体可与hepcidin或hepcidin激动剂联用。一种主要产生于肝的循环多肽hepcidin由于其诱导ferroportin(一种位于吸收性肠细胞、肝细胞和巨噬细胞的铁输出蛋白)降解的能力而被视为铁代谢的主要调节剂。一般地说,hepcidin降低胞外铁的可用性,因此hepcidin激动剂在治疗无效红细胞生成中可能是有益的[参见例如Nemeth(2010)Adv Hematol 2010:750643]。这个看法得到得到β-珠蛋白生成障碍性贫血小鼠模型中hepcidin表达的有益作用的支持[Gardenghi等(2010)J Clin Invest120:4466-4477]。

[0582] 任选与EPO受体激活物组合的本公开内容的一种或多种TGF-β超家族异多聚体复合体还可适用于治疗无序RBC成熟性贫血,其特征部分在于不够大(小红细胞)、过大(大红细胞)、畸形或异常着色(血蛋白过少)的RBC。

[0583] 在某些实施方案中,本公开内容提供通过给予个体治疗有效量的本公开内容的一种或多种TGF-β超家族异多聚体复合体和EPO受体激活物治疗或预防有需要的个体的贫血的方法。在某些实施方案中,本公开内容的一种或多种TGF-β超家族异多聚体复合体可与EPO受体激活物联用以降低易受EPO不良反应影响的患者中这些激活物的所需剂量。这些方法可用于患者的治疗性或预防性治疗。

[0584] 本公开内容的一种或多种TGF-β超家族异多聚体复合体可与EPO受体激活物联用以实现红细胞的增加,特别在EPO受体激活物较低的剂量范围内。这在降低已知的脱靶作用和与高剂量EPO受体激活物有关的风险中可能是有益的。EPO的初级不良反应包括例如血细胞比容或血红蛋白水平的过度增加和红细胞增多。血细胞比容水平升高可导致高血压(更具体地说高血压加重)和血管血栓形成。已报道的EPO的其它不良反应,其中一些与高血压

有关,有头痛、流感样综合征、分流的阻断、因血栓形成引起的心肌梗死和脑惊厥、高血压脑病和红细胞血细胞发育不全。参见例如Singibarti(1994)J.Clin Investig 72(suppl 6),S36-S43;Horl等(2000)Nephrol Dial Transplant 15(suppl 4),51-56;Delanty等(1997)Neurology 49,686-689;以及Bunn(2002)N Engl J Med 346(7),522-523)。

[0585] 如果本公开内容的TGF-β超家族异多聚体复合体通过不同于EPO的机制起作用,则这些拮抗剂可用于在对EPO反应不良的患者中提高红细胞和血红蛋白水平。例如本公开内容的TGF-β超家族异多聚体复合体可能有利于这样的患者,其中给予该患者正常到剂量升高的EPO(>300IU/kg/周)无法使血红蛋白水平上升至目标水平。对EPO反应不足的患者可见于所有类型的贫血中,但在癌症患者和晚期肾病患者中特别频繁地观察到更高数量的非应答者。对EPO反应不足可以是组成型(在第一次用EPO治疗时即观察到)或获得性的(在用EPO反复治疗后观察到)。

[0586] 在某些实施方案中,本公开提供了用于通过测量患者体内一项或多项血液学参数来管理患者的方法,所述患者已经用(或是作为候选者将用)本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗。所述血液学参数可用于评价用于患者(其为待用本公开内容的拮抗剂治疗的候选者)的合适剂量、用于监测治疗期间的血液学参数、用于评价在用本公开内容的一种或多种拮抗剂治疗期间是否调整剂量和/或用于评价本公开内容的一种或多种拮抗剂的合适的维持剂量。如果所述血液学参数中的一项或多项在正常水平之外,则本公开内容的一种或多种TGF-β超家族异多聚体复合体的给药可减少、推迟或终止。

[0587] 可采用本领域公认的方法,按照本文提供的方法,测量的血液学参数包括例如红细胞水平、血压、铁储存以及与红细胞水平升高相关的存在于体液中的其它作用剂。这类参数可使用患者血样测定。红细胞水平、血红蛋白水平和/或血细胞比容水平的升高可能引起血压升高。

[0588] 在一个实施方案中,如果作为用本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗的候选者的患者中一项或多项血液学参数在正常范围以外或在正常的高位,则开始给予本公开内容的一种或多种TGF-β超家族异多聚体复合体可能被推迟直到所述血液学参数自然地或通过治疗干预回复正常或可接受水平。例如,如果候选患者有高血压或高血压前期,则患者可用降压药治疗以降低患者的血压。可使用适于各个患者状况的任何降压药,包括例如利尿药、肾上腺素能抑制剂(包括α阻断剂和β阻断剂)、血管扩张药、钙通道阻断剂、血管紧张素转化酶(ACE)抑制剂或血管紧张素II受体阻断剂。血压可备选地使用饮食和运动方案治疗。同样,如果候选患者具有低于正常的或在正常低位的铁储存,则患者可用合适的饮食和/或铁补充剂方案治疗直到患者的铁储存回复到正常或可接受水平。对于具有高于正常的红细胞水平和/或血红蛋白水平的患者而言,则给予本公开内容的一种或多种TGF-β超家族异多聚体复合体可能被推迟直到所述水平回复到正常或可接受水平。

[0589] 在某些实施方案中,如果作为用本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗的候选者的患者中一项或多项血液学参数在正常范围以外或在正常的高位,则开始给药可能不被推迟。然而,本公开内容的一种或多种TGF-β超家族异多聚体复合体的给药剂量或频率可能被设定为这样的量,其将降低在给予本公开内容的一种或多种TGF-β超家族异多聚体复合体时产生的不可接受的血液参数升高的风险。或者,可开发针对所述患者的治疗方案,其将本公开内容的一种或多种TGF-β超家族异多聚体复合体与解决不期望

的血液学参数水平的治疗剂组合。例如，如果患者的血压升高，则可设计涉及给予本公开内容的一种或多种TGF-β超家族异多聚体复合体和降压药的治疗方案。对于具有低于所需铁储存的患者，可开发这样的治疗方案，其包括本公开内容的一种或多种TGF-β超家族异多聚体复合体和铁补充的治疗方案。

[0590] 在一个实施方案中，可针对作为待用本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗的候选者的患者和针对该患者基于基线值确立的合适给药方案，确立一项或多项血液学参数的基线参数。或者，基于患者病史确立的基线参数可用于了解用于患者的合适给药方案。例如，如果健康患者的已确立的基线血压读数高于规定的正常范围，则在用本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗前，可能不必将所述患者的血压降到被认为对于普通人群是正常的范围。在用本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗之前患者的一项或多项血液学参数的基线值还可用作相关比较值用于监测在用本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗期间所述血液学参数的任何变化。

[0591] 在某些实施方案中，测量用本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗的患者的一项或多项血液学参数。血液学参数可用于在治疗期间监测所述患者并允许调整或终止给予本公开内容的一种或多种TGF-β超家族异多聚体复合体或额外给予另一种治疗剂。例如，如果给予本公开内容的一种或多种TGF-β超家族异多聚体复合体导致血压、红细胞水平或血红蛋白水平升高或铁储存减少，则可减少本公开内容的一种或多种TGF-β超家族异多聚体复合体的剂量或频率以降低本公开内容的一种或多种TGF-β超家族异多聚体复合体对一项或多项血液学参数的影响。如果给予本公开内容的一种或多种TGF-β超家族异多聚体复合体导致一项或多项血液学参数出现对患者不利的变化，则可暂时终止给予本公开内容的一种或多种TGF-β超家族异多聚体复合体直到血液学参数回复到可接受的水平，或可永久终止给药。同样，如果在减少本公开内容的一种或多种TGF-β超家族异多聚体复合体的剂量或频率之后，一项或多项血液学参数仍未回复到可接受的范围内，则可终止给药。作为减少或终止给予本公开内容的一种或多种TGF-β超家族异多聚体复合体的备选方式或除此之外，可向所述患者给予应对不期望的血液学参数水平的其它治疗剂，例如降压药或铁补充剂。例如，如果正在接受本公开内容的一种或多种TGF-β超家族异多聚体复合体治疗的患者的血压升高，则可以相同水平继续给予本公开内容的一种或多种TGF-β超家族异多聚体复合体并向治疗方案中加入降压药、可减少给予本公开内容的一种或多种TGF-β超家族异多聚体复合体(例如量和/或频率)并向治疗方案中加入降压药、或可终止给予本公开内容的一种或多种TGF-β超家族异多聚体复合体并可用降压药治疗患者。

[0592] 6.药物组合物

[0593] 在某些方面，本公开内容的TGF-β超家族异多聚体复合体可以单独给予或作为药物制剂(亦称为治疗组合物或药物组合物)的组分给予。药物制剂是指呈如此形式使得允许其中所含活性成分的生物活性起效并且不含对将向其给予制剂的受试者具有不可接受的毒性的其它组分的制品。可配制主题化合物以用于人或兽医用药的任何便利方式给药。例如，本公开内容的一种或多种作用剂可与药学上可接受的载体一起配制。药学上可接受的载体是指药物制剂中活性成分之外的成分，其通常对受试者是无毒的。药学上可接受的载体包括但不限于：缓冲剂、赋形剂、稳定剂或防腐剂。通常，用于本公开内容的药物制剂当给

予受试者时是无热原的、生理学上可接受的形式。可任选包括在上述制剂中的本文所述作用剂以外的治疗有益的作用剂，在本公开内容的方法中可与主题作用剂组合给予。

[0594] 在某些实施方案中，组合物将肠胃外给予[例如通过静脉内(I.V.)注射、动脉内注射、骨内注射、肌内注射、鞘内注射、皮下注射或皮内注射]。适用于肠胃外给药的药物组合物可包含本公开内容的一种或多种作用剂与一种或多种药学上可接受的无菌等渗水溶液或非水溶液、分散液、悬浮液或乳液或无菌粉末(其可在临用前重配成无菌注射剂或分散剂)的组合。注射注射剂或分散剂可含有抗氧化剂、缓冲剂、抑菌剂、助悬剂、增稠剂或使制剂与预定接受者的血液等渗的溶质。可用于本公开内容的药物制剂中的合适的水性或非水性载体的实例包括：水、乙醇、多元醇(例如甘油、丙二醇、聚乙二醇等)、植物油(例如橄榄油)、注射用有机酯(例如油酸乙酯)及其合适的混合物。例如可通过使用包衣材料(例如卵磷脂)、通过在分散剂的情况下保持所需的粒径以及通过使用表面活性剂来保持适当的流动性。

[0595] 在一些实施方案中，本公开内容的治疗方法包括从植入物或装置全身或局部给予药物组合物。另外，药物组合物可以一定形式包封或注射用于递送至靶组织部位(例如骨髓或肌肉)。在某些实施方案中，本公开内容的组合物可包含能够将本公开内容的一种或多种作用剂递送至靶组织部位(例如骨髓或肌肉)的基质，为发育中的组织提供结构并最好能够被身体再吸收。例如，所述基质可提供本公开内容的一种或多种作用剂的缓慢释放。这类基质可由目前用于其它植入的医疗应用的材料形成。

[0596] 基质材料的选择基于以下一项或多项：生物相容性、生物降解能力、机械性能、美容外观和界面性质。主题组合物的具体应用将界定适当的制剂。用于组合物的可能基质可以是生物可降解的和化学成分确定的硫酸钙、磷酸三钙、羟磷灰石、聚乳酸和聚酐。其它可能的材料是生物可降解的和生物学成分清晰确定的，例如骨或真皮胶原。其它基质由纯的蛋白质或胞外基质组分组成。其它可能的基质是非生物可降解的和化学成分确定的，例如烧结羟磷灰石、生物玻璃、铝酸盐或其它陶瓷。基质可由任何上述材料类型的组合组成，例如聚乳酸和羟磷灰石或胶原和磷酸三钙。生物陶瓷在组成方面可改变(例如钙-铝酸盐-磷酸盐)，并进行加工以改变孔径、颗粒大小、颗粒形状和生物降解能力的一项或多项。

[0597] 在某些实施方案中，本公开内容的药物组合物可局部给予。“局部施用”或“局部”意指药物组合物与身体表面(包括例如皮肤、伤口部位和粘膜)接触。局部药物组合物可具有不同的应用形式，通常包含含药层，其适于在局部给予组合物时置于组织附近或与组织直接接触。适于局部给药的药物组合物可包含本公开内容的一种或多种TGFβ超家族I型和/或II型受体多肽复合体，组合配制成液体制剂、凝胶剂、乳膏剂、洗剂、软膏剂、泡沫剂、糊剂、擦粉剂(putty)、半固体剂或固体剂。可通过将组合物在靶组织上涂撒、喷雾、涂抹、涂敷或铺展，施用液体剂、凝胶剂、乳膏剂、洗剂、软膏剂、泡沫剂、糊剂或擦粉形式中的组合物。还可将组合物浸入无菌敷料剂、透皮贴剂、硬膏和绷带中。擦粉剂、半固体剂或固体剂形式的组合物可能是可变形的。它们可以是弹性或无弹性的(例如柔性或刚性)。在某些方面，组合物形成复合物的一部分，并可包括纤维、微粒或具有相同或不同组合物的多个层。

[0598] 液体形式的局部组合物可包括药学上可接受的溶液剂、乳剂、微乳剂和混悬剂。除活性成分以外，液体剂型可含有本领域常用的惰性稀释剂，包括例如水或其它溶剂、增溶剂和/或乳化剂[例如乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苄醇、苯甲酸乙酯、丙二醇或1,3-丁

二醇、油(例如棉籽油、花生油、玉米油、胚芽油、橄榄油、蓖麻油和芝麻油)、甘油、四氢糠醇、聚乙二醇、失水山梨糖醇的脂肪酸酯及其混合物]。

[0599] 局部凝胶剂、乳膏剂、洗剂、软膏剂、半固体或固体组合物可包括一种或多种增稠剂,例如多糖、合成聚合物或基于蛋白质的聚合物。在本发明的一个实施方案中,本文的胶凝剂是适当无毒的且提供所需粘度的胶凝剂。增稠剂可包括以下的聚合物、共聚物和单体:乙烯吡咯烷酮、甲基丙烯酰胺、丙烯酰胺N-乙烯基咪唑、羧基乙烯基、乙烯基酯、乙烯基醚、硅酮、聚氧化乙烯、聚乙二醇、乙烯基醇、丙烯酸钠、丙烯酸盐、马来酸、NN-二甲基丙烯酰胺、双丙酮丙烯酰胺、丙烯酰胺、丙烯酰吗啉、普朗尼克酸、胶原、聚丙烯酰胺、聚丙烯酸酯、聚乙烯醇、聚亚乙烯基、聚硅酸乙烯酯、被糖(例如蔗糖、葡萄糖、氨基葡萄糖、半乳糖、海藻糖、甘露糖或乳糖)取代的聚丙烯酸酯、丙烯酰胺基丙烷磺酸、正硅酸四甲氧基酯、正硅酸甲基三甲氧基酯、正硅酸烷氧基酯、正硅酸三烷氧基酯、二醇、丙二醇、甘油、多糖、藻酸盐、葡聚糖、环糊精、纤维素、改性纤维素、氧化纤维素、脱乙酰壳多糖、壳多糖、瓜尔胶、角叉菜胶、透明质酸、菊糖、淀粉、改性淀粉、琼脂糖、甲基纤维素、植物树胶、乙酰透明质酸、水凝胶、明胶、糖胺聚糖、羧甲基纤维素、羟乙基纤维素、羟丙基甲基纤维素、果胶、低甲氧基果胶、交联葡聚糖、淀粉-丙烯腈接枝共聚物、淀粉聚丙烯酸钠、甲基丙烯酸羟乙酯、丙烯酸羟乙酯、聚亚乙烯、聚乙基乙烯基醚、聚甲基丙烯酸甲酯、聚苯乙烯、聚氨酯、聚链烷酸酯、聚乳酸、聚乳酸酯、聚(3-羟基丁酸酯)、磺化水凝胶、AMPS(2-丙烯酰氨基-2-甲基-1-丙磺酸)、SEM(甲基丙烯酸磺乙酯)、SPM(甲基丙烯酸磺丙酯)、SPA(丙烯酸磺丙酯)、N,N-二甲基-N-甲基丙烯酰氧基乙基-N-(3-磺丙基)铵甜菜碱、甲基丙烯酸酰氨基丙基-二甲基铵磺基甜菜碱、SPI(衣康酸-双(1-丙基磺酸-3)酯二钾盐)、衣康酸、AMBC(3-丙烯酰氨基-3-甲基丁酸)、β-羧基乙基丙烯酸酯(丙烯酸二聚体)和马来酸酐-甲基乙烯基醚聚合物、其衍生物、其盐、其酸及其组合。在某些实施方案中,本公开内容的药物组合物可口服给予,例如为胶囊剂、扁囊剂、丸剂、片剂、锭剂(使用调味基料例如蔗糖和阿拉伯树胶或西黄蓍胶)、散剂、颗粒剂、水性或非水性液体中的溶液剂或混悬剂、水包油或油包水液体乳剂或酏剂或糖浆剂或含锭剂(使用惰性基料,例如明胶和甘油、或蔗糖和阿拉伯树胶)和/或漱口剂的形式,各含有预定量的本公开内容的化合物和任选一种或多种其它活性成分。本公开内容的化合物和任选一种或多种其它活性成分还可作为大丸剂、药糖剂或糊剂给予。

[0600] 在用于口服给药的固体剂型(例如胶囊剂、片剂、丸剂、糖衣丸、散剂和颗粒剂)中,可将本公开内容的一种或多种化合物与包括例如以下的一种或多种药学上可接受的载体混合:柠檬酸钠、磷酸二钙、填充剂或增充剂(例如淀粉、乳糖、蔗糖、葡萄糖、甘露醇和/或硅酸)、粘合剂(例如羧甲基纤维素、藻酸盐、明胶、聚乙烯吡咯烷酮、蔗糖和/或阿拉伯胶)、湿润剂(例如甘油)、崩解剂(例如琼脂、碳酸钙、马铃薯或木薯淀粉、藻酸、硅酸盐和碳酸钠)、溶液阻滞剂(例如石蜡)、吸收促进剂(例如季铵化合物)、润湿剂(例如鲸蜡醇和单硬脂酸甘油酯)、吸收剂(例如高岭土和膨润土)、润滑剂(例如滑石粉、硬脂酸钙、硬脂酸镁、固态聚乙二醇、十二烷基硫酸钠)、着色剂及其混合物。在胶囊剂、片剂和丸剂的情况下,药物制剂(组合物)还可包含缓冲剂。在使用包括乳糖以及高分子量聚乙二醇等在内的一种或多种赋形剂的软充填和硬充填明胶胶囊剂中,相似类型的固体组合物也可用作填充剂。

[0601] 用于药物组合物的口服给药的液体剂型可包括药学上可接受的乳剂、微乳剂、溶液剂、混悬剂、糖浆剂和酏剂。除活性成分以外,液体剂型可含有常用于本领域的惰性稀释

剂，包括例如水或其它溶剂、增溶剂和乳化剂[例如乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苯甲醇、苯甲酸苄酯、丙二醇、1,3-丁二醇、油(例如棉籽油、花生油、玉米油、胚芽油、橄榄油、蓖麻油和芝麻油)、甘油、四氢糠醇、聚乙二醇和失水山梨糖醇的脂肪酸酯及其混合物]。除惰性稀释剂以外，口服制剂还可包含辅料，包括例如润湿剂、乳化剂和助悬剂、甜味剂、矫味剂、着色剂、芳香剂和防腐剂及其组合。

[0602] 除活性化合物以外，混悬剂可含有助悬剂，包括例如乙氧基化异十八醇、聚氧乙烯山梨糖醇和失水山梨糖醇酯、微晶纤维素、偏氢氧化铝(aluminum metahydroxide)、膨润土、琼脂和西黄蓍胶及其组合。

[0603] 可通过加入多种抗细菌剂和抗真菌剂，包括例如对羟基苯甲酸酯、三氯叔丁醇、苯酚、山梨酸等，来确保防止微生物的作用。

[0604] 在某些实施方案中，组合物中还可能需要包含等渗剂，包括例如糖、氯化钠。另外，可通过加入延迟吸收的物质(例如单硬脂酸铝和明胶)，使可注射药物形式的吸收延长。

[0605] 要了解，主治医师可在考虑修正本公开内容的一种或多种作用剂的作用的各种因素后，确定剂量方案。在促进红细胞形成的TGF-β超家族异多聚体复合体的情况下，各种因素可包括但不限于患者的红细胞计数、血红蛋白水平、所需的目标红细胞计数、患者年龄、患者性别、患者饮食、可能造成红细胞水平下降的任何疾病的严重程度、给药时间和其它临床因素。向最终的组合物中加入其它已知活性剂也可能影响剂量。可通过定期评价红细胞水平、血红蛋白水平、网织红细胞水平和造血过程的其它指示物的一项或多项来监测过程。

[0606] 在某些实施方案中，本公开内容还提供用于体内产生本公开内容的一种或多种作用剂的基因疗法。这类疗法可通过将作用剂序列引入受累于上文所列病症的细胞或组织中，来达到其治疗效果。可使用重组表达载体(例如嵌合病毒或胶体分散系统)实现作用剂序列的递送。本公开内容的一种或多种作用剂序列优选的治疗递送是使用靶向脂质体。

[0607] 可用于本文教导的基因疗法的各种病毒载体包括腺病毒、疱疹病毒、痘苗病毒或优选RNA病毒(例如反转录病毒)。反转录病毒载体是鼠或禽反转录病毒的衍生物。其中可插入单一外源基因的反转录病毒载体的实例包括但不限于：莫洛尼鼠白血病病毒(MoMuLV)、Harvey鼠肉瘤病毒(HaMuSV)、鼠乳腺肿瘤病毒(MuMTV)和劳斯肉瘤病毒(Rous sarcoma virus,RSV)。许多其它的反转录病毒载体可掺入多个基因。所有的这些载体可转移或整合选择标记的基因，使得可鉴定和产生转导细胞。可通过连接例如糖、糖脂或蛋白质，使反转录病毒载体具有靶标特异性。通过使用抗体来完成优选的打靶。本领域的技术人员应认识到，可将特异性多核苷酸序列插入反转录病毒基因组或与病毒包膜连接，以供含有本公开内容的一种或多种作用剂的反转录病毒载体的靶标特异性递送。

[0608] 或者，可通过常规磷酸钙转染，将组织培养细胞用编码反转录病毒结构基因gag、pol和env的质粒直接转染。然后将这些细胞用含有目标基因的载体质粒转染。所得细胞释放反转录病毒载体到培养基中。

[0609] 本公开内容的一种或多种作用剂的另一种靶定递送系统是胶体分散系统。胶体分散系统包括大分子复合体、纳米囊、微球、珠粒和脂质型系统，包括水包油乳液、微团、混合微团和脂质体。在某些实施方案中，优选的本公开内容的胶体系统是脂质体。脂质体是可体外和体内用作递送载体的人工膜囊泡。RNA、DNA和完整病毒体可包封在水性内部，并以生物活性形式递送至细胞。参见例如Fraley等(1981)Trends Biochem.Sci.,6:77。使用脂质体

载体的有效基因转移方法是本领域已知的，参见例如Mannino等(1988)Biotechniques,6:682,1988。

[0610] 脂质体的组成一般是磷脂的组合，磷脂可包括类固醇(例如胆固醇)。脂质体的物理特性取决于pH、离子强度和二价阳离子的存在情况。还可使用的其它磷脂或其它脂质包括例如磷脂酰化合物，例如磷脂酰甘油、磷脂酰胆碱、磷脂酰丝氨酸、磷脂酰乙醇胺、鞘脂、脑苷脂和神经节苷脂。说明性的磷脂包括蛋黄磷脂酰胆碱(egg phosphatidylcholine)、二棕榈酰磷脂酰胆碱和二硬脂酰磷脂酰胆碱。根据例如器官特异性、细胞特异性和细胞器特异性靶定脂质体也是可行的，并且是本领域已知的。

[0611] 实例

[0612] 现对本发明进行了大致描述，通过参照下列实施例可更容易地理解本发明，只包括用于说明本发明的某些实施方案和实施方案的目的的实施例，并无意限制本发明。

[0613] 实施例1.ActRIIB-Fc:ALK4-Fc异二聚体的产生

[0614] 申请人构建了包含人ActRIIB和人ALK4的胞外域(其各自分别与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIB-Fc:ALK4-Fc异聚体复合体。各个构建体分别被称为ActRIIB-Fc融合多肽和ALK4-Fc融合多肽，下面提供每个的序列。

[0615] 与ActRIIB-Fc或ALK4-Fc同二聚体复合体不同，用于促进ActRIIB-Fc:ALK4-Fc异聚体复合体形成的方法是将变化引入Fc结构域的氨基酸序列中以指导不对称异聚体复合体的形成。本公开内容描述了使用Fc结构域制备不对称相互作用对的许多不同的方法。

[0616] 在一种方法中，分别在SEQ ID NO:100-102和104-106的ActRIIB-Fc和ALK4-Fc多肽序列中说明的，改变一个Fc结构域以在相互作用面上引入阳离子氨基酸，同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。ActRIIB-Fc融合多肽和ALK4-Fc融合多肽各自使用组织纤溶酶原激活物(TPA)前导序列:MDAMKRGLCCVLLLCGAVFVSP(SEQ ID NO:98)。

[0617] ActRIIB-Fc多肽序列(SEQ ID NO:100)见下：

[0618]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
```

[0619]
```
251  APIEKTISKA KGQPREPQVY TLPPSRKEMT KNQVSLTCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK          (SEQ ID NO: 100)
```

[0620] 前导(信号)序列和接头用下划线表示。为了促进ActRIIB-Fc:ALK4-Fc异二聚体而非可能的同二聚体复合体任一个的形成，可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示ActRIIB融合蛋白的Fc结构域中。可任选提供从C端除去赖氨酸(K)的SEQ ID NO:100的氨基酸序列。

[0621] 该ActRIIB-Fc融合蛋白由下列核酸序列(SEQ ID NO:101)编码：

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

[0622]

[0623] 成熟的ActRIIB-Fc融合多肽(SEQ ID NO:102)如下,并且可任选提供从C端脱去赖氨酸的多肽。

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

[0624]

[0625] ALK4-Fc融合多肽的互补形式(SEQ ID NO:104)如下:

[0626]

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

[0627] 前导序列和接头序列用下划线表示。为了引导用上述SEQ ID NO:100和102的ActRIIB-Fc融合多肽形成异二聚体,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示ALK4-Fc融合多肽的Fc结构域中。可任选提供在C端加入赖氨酸的SEQ ID NO:104的氨基酸序列。

[0628] 该ALK4-Fc融合蛋白由下列核酸(SEQ ID NO:105)编码:

[0629]

```
   1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51 AGTCTTCGTT TCGCCCGGCG CCTCCGGGCC CCGGGGGGTC CAGGCTCTGC
 101 TGTGTGCGTG CACCAGCTGC CTCCAGGCCA ACTACACGTG TGAGACAGAT
 151 GGGGCCTGCA TGGTTTCCAT TTTCAATCTG GATGGGATGG AGCACCATGT
 201 GCGCACCTGC ATCCCCAAAG TGGAGCTGGT CCCTGCCGGG AAGCCCTTCT
 251 ACTGCCTGAG CTCGGAGGAC CTGCGCAACA CCCACTGCTG CTACACTGAC
 301 TACTGCAACA GGATCGACTT GAGGGTGCCC AGTGGTCACC TCAAGGAGCC
 351 TGAGCACCCG TCCATGTGGG GCCCGGTGGA GACCGGTGGT GGAACTCACA
 401 CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
 451 CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
 501 GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
 551 TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
 601 CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
 651 CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA
 701 ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
 751 CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGAGGAGAT
 801 GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA
 851 GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC
 901 GACACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTATAG
 951 CGACCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001 GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
1051 TCCCTGTCTC CGGGT      (SEQ ID NO:105)
```

[0631] 成熟的ALK4-Fc融合蛋白序列(SEQ ID NO:106)如下,并可任选提供C端加入赖氨酸的序列。

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

[0633] SEQ ID NO:102和SEQ ID NO:106的ActRIIB-Fc和ALK4-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIB-Fc:ALK4-Fc的异聚体复合体。

[0634] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,改变Fc结构域以引入分别如SEQ ID NO:401-402和403-404的ActRIIB-Fc和ALK4-Fc多肽序列所示的互补疏水相互作用和其它分子间二硫键。ActRIIB-Fc融合多肽和ALK4-Fc融合多肽各自使用组织纤溶酶原激活物(TPA)前导序列:MDAMKRGLCCVLLLCGAVFVSP(SEQ ID NO:98)。

[0635] ActRIIB-Fc多肽序列(SEQ ID NO:401)见下:

[0636]
```
   1 MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
  51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
 101 ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
```

[0637]
```
251 APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPSDIAV
301 EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQK SLSLSPGK          (SEQ ID NO: 401)
```

[0638] 前导(信号)序列和接头序列用下划线表示。为了促进ActRIIB-Fc:ALK4-Fc异二聚体而非可能的同二聚体复合体任一个的形成,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:401的氨基酸序列。

[0639] 成熟的ActRIIB-Fc融合多肽如下:

[0640]
```
   1 GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
  51 IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
 101 GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151 RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC
251 REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK     (SEQ ID NO:402)
```

[0641] ALK4-Fc融合多肽的互补形式(SEQ ID NO:403)如下,并可任选提供从C端脱去赖氨酸的多肽。

[0642]
```
  1 MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 51 GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101 YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201 REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251 QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY
301 KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351 SLSPGK    (SEQ ID NO: 403)
```

[0643] 前导序列和接头用下划线表示。为了引导用上文SEQ ID NO:401和402的ActRIIB-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入上文双下划线所示ALK4融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:403的氨基酸序列。

[0644] 成熟的ALK4-Fc融合蛋白序列如下并可任选以从C端脱去赖氨酸的序列提供。

[0645]
```
  1 SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
 51 ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101 PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
251 SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS
301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 404)
```

[0646] SEQ ID NO:402和SEQ ID NO:404的ActRIIB-Fc和ALK4-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIB-Fc:ALK4-Fc的异聚体复合体。

[0647] 不同的ActRIIB-Fc:ALK4-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0648] 实施例2.与ActRIIB-Fc同二聚体和ALK4-Fc同二聚体相比ActRIIB-Fc:ALK4-Fc异二聚体的配体结合特征

[0649] 采用基于Biacore™的结合测定法以比较上文所述ActRIIB-Fc:ALK4-Fc异二聚体复合体与ActRIIB-Fc和ALK4-Fc同二聚体复合体的配体结合选择性。使用抗Fc抗体将ActRIIB-Fc:ALK4-Fc异二聚体、ActRIIB-Fc同二聚体和ALK4-Fc同二聚体独立俘获在系统中。注入配体，并使之在俘获的受体蛋白上流过。结果概括于下表，其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影表示。

[0650] 配体解离速率是评价配体阱的特别重要的参数。体内给予的可溶性受体-Fc蛋白与配体的天然受体不断竞争。当TGFβ超家族的内源配体典型地在细胞表面与关联受体结合时，多步骤信号转导过程被触发，其在分子时间尺度上相对慢。天然受体部分从配体上慢慢解离，因为从配体结合事件产生胞内信号需要相当的时间。对于与配体有效竞争的可溶性受体-Fc蛋白，其复合体与配体的解离速率需要相当于或慢于配体复合体与天然受体的解离速率。配体结合是一个动态过程，一部分的配体将总是处于未结合形式，因此受体-Fc蛋白尽可能久地俘获靶配体的剂量在治疗上是重要的。改变有利于更多俘获配体的结合平衡的一种方法是提高抑制剂的浓度(剂量水平)，然而这可能产生降低耐受性和安全性的脱靶

作用。优选的方法是使用具有较慢配体解离速率(较长俘获时间)与配体结合选择性组合的抑制剂,以较低的抑制剂浓度实现配体拮抗作用的有效水平。

[0651]

与 ActRIIB-Fc 同二聚体和 ALK4-Fc 同二聚体相比
ActRIIB-Fc:ALK4-Fc 异二聚体的配体结合特征

配体	ActRIIB-Fc 同二聚体			ALK4-Fc 同二聚体			ActRIIB-Fc:ALK4-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
激活素 A	1.2×10^7	2.3×10^{-4}	19	5.8×10^5	1.2×10^{-2}	20000	1.3×10^7	1.5×10^{-4}	12
激活素 B	5.1×10^6	1.0×10^{-4}	20	无结合			7.1×10^6	4.0×10^{-5}	6
BMP6	3.2×10^7	6.8×10^{-3}	190	---			2.0×10^6	5.5×10^{-3}	2700
BMP9	1.4×10^7	1.1×10^{-3}	77	---			瞬时*		3400
BMP10	2.3×10^7	2.6×10^{-4}	11	---			5.6×10^7	4.1×10^{-3}	74
GDF3	1.4×10^6	2.2×10^{-3}	1500	---			3.4×10^6	1.7×10^{-2}	4900
GDF8	8.3×10^5	2.3×10^{-4}	280	1.3×10^5	1.9×10^{-3}	15000†	3.9×10^5	2.1×10^{-4}	550
GDF11	5.0×10^7	1.1×10^{-4}	2	5.0×10^6	4.8×10^{-3}	270†	3.8×10^7	1.1×10^{-4}	3

* 因相互作用的瞬时性质而不确定
† 极低信号
--- 未测

[0652] 这些比较结合数据表明相对于ActRIIB-Fc或ALK4-Fc同二聚体,ActRIIB-Fc:ALK4-Fc异二聚体的结合特征/选择性改变。ActRIIB-Fc:ALK4-Fc异二聚体显示与任一种同二聚体相比与激活素B的结合增强,与用ActRIIB-Fc同二聚体观察到的一样保持与激活素A、GDF8和GDF11牢固结合,且显示与BMP9、BMP10和GDF3的结合大大减弱。具体地讲,BMP9显示针对ActRIIB-Fc:ALK4-Fc异二聚体低或无可观察的亲和力,而该配体与ActRIIB-Fc同二聚体牢固结合。像ActRIIB-Fc同二聚体一样,异二聚体保持与BMP6的中等水平结合。参见图6。

[0653] 这些结果因此表明与ActRIIB-Fc同二聚体相比,ActRIIB-Fc:ALK4-Fc异二聚体是更高选择性的激活素A、激活素B、GDF8和GDF11的拮抗剂。因此,在其中这类选择性拮抗作用是有利的某些应用中,ActRIIB-Fc:ALK4-Fc异二聚体将比ActRIIB-Fc同二聚体更有用。实例包括其中需要保持激活素A、激活素B、激活素AC、GDF8和GDF11的一种或多种的拮抗作用但使BMP9、BMP10和BMP6的一种或多种的拮抗作用减到最小的治疗应用。

[0654] 实施例3.小鼠中与ActRIIB-Fc同二聚体相比ActRIIB-Fc:ALK4-Fc异二聚体的活性特征

[0655] 在小鼠中测试同二聚体和异二聚体复合体以研究其体内活性特征的差异。从约10周龄(n＝9只小鼠/周)开始,每周两次皮下给予野生型C57BL/6小鼠ActRIIB-Fc同二聚体(10mg/kg)、ActRIIB-Fc:ALK4-Fc异二聚体(3或10mg/kg)或溶媒(磷酸缓冲盐水,PBS)达4周。如通过表面等离子体共振测定的,因其在无细胞条件下无法以高亲和力结合配体,故未体内测定ALK4-Fc同二聚体。研究终点包括:在基线和研究完成(4周)通过核磁共振(NMR)测定的体重、总瘦体质量(total lean mass)和总脂肪质量;在基线和4周通过双能X光吸光测定法(DEXA)测定的总骨矿物质密度;以及在4周测定的腓肠肌、股直肌和胸肌重量。

[0656]

野生型小鼠中 ActRIIB-Fc 和 ALK4-Fc 复合体的活性				
终点 (4 wk)	溶媒	ActRIIB-Fc 同二聚体 10 mg/kg	ActRIIB-Fc:ALK4-Fc 异二聚体	
			10 mg/kg	3 mg/kg
体重自基线的变化	↑15%	↑38% **	↑41% **	↑33% **
总瘦体质量自基线的变化	↓1%	↑5% **	↑5% **	↑5% **
总脂肪质量自基线的变化	↑5%	↓36% **	↓35% **	↓35% **

[0657]

总骨矿物质密度自基线的变化	↑8%	↑14% *	↑12% *	↑11%
腓肠肌重量†	23	36 **	35 **	30 **
股直肌重量†	11.5	17 **	16 **	14 **
胸肌重量†	15	23 **	28 **	23 **
*P< 0.05 相对于溶媒 **P< 0.01 相对于溶媒 †归一化至股骨长度(mg/mm)以控制体格大小的综合左和右肌肉重量				

[0658] 研究结果概括于上表中。正如所预期的,ActRIIB-Fc同二聚体引起身体组成的显明变化,许多与GDF8和激活素抑制的已知作用一致。与溶媒治疗对照相比,用ActRIIB-Fc同二聚体治疗野生型小鼠在研究过程中体重增加超过两倍。与溶媒相比,伴随这种净体重增加的是总瘦体质量和总骨矿物质密度的显著增加,以及总脂肪质量的显著降低。应认识到,瘦组织和脂肪组织的归一化(百分比型)变化不同于其相应的绝对变化,因为瘦体质量(小鼠中通常约为体重的70%)远大于脂肪质量(通常约为体重的10%)。检查各个骨骼肌(包括腓肠肌、股直肌和胸肌),用ActRIIB-Fc同二聚体治疗过程中,与溶媒对照相比,所有的重量都显著增加。

[0659] ActRIIB-Fc:ALK4-Fc异二聚体产生惊人地类似于ActRIIB-Fc同二聚体的某些作用,尽管两种复合体的差异性配体选择性。如上表所示,对于所列的所有终点,小鼠以10mg/

kg的剂量水平用ActRIIB-Fc:ALK4-Fc异二聚体治疗比得上、几乎比得上或超过相同剂量水平的ActRIIB-Fc同二聚体的作用。对于几项终点，与10mg/kg相比，3mg/kg的ActRIIB-Fc:ALK4-Fc异二聚体的作用轻微减弱，因此提供剂量-作用关系的证据。

[0660] 因此，ActRIIB-Fc:ALK4-Fc异二聚体对骨骼肌和骨发挥有益的合成代谢作用，并对脂肪组织发挥分解代谢作用，与ActRIIB-Fc同二聚体的作用非常类似。然而，与ActRIIB同二聚体不同，ActRIIB-Fc:ALK4-Fc异二聚体对BMP9和BMP10只显示低亲和力或瞬时结合，因此不会同时抑制由所述配体介导的过程，例如血管生成。这种新的选择性在例如治疗需要对肌肉和骨的刺激作用和对脂肪的抑制作用，但不需要改变血管生成的患者中将是有用的。

[0661] 实施例4.ActRIIB-Fc:ALK3-Fc异二聚体的产生

[0662] 申请人构建了包含人ActRIIB和人ALK3的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIB-Fc:ALK3-Fc异聚体复合体。各个构建体分别被称为ActRIIB-Fc和ALK3-Fc。

[0663] 通过类似于实施例1所述方法可引导异聚体ActRIIB-Fc:ALK3-Fc的形成。

[0664] 在第一种方法中，在上文实施例1中以SEQ ID NO:100-102提供ActRIIB-Fc融合蛋白的多肽序列和其编码核酸序列。

[0665] 互补ALK3-Fc融合蛋白使用TPA前导序列，并列举如下：

[0666]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51  LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
101  CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
151  SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
301  TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF LYSDLTVDKS
351  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G  (SEQ ID NO: 115)
```

[0667] 前导序列和接头序列用下划线表示。为了促进ActRIIB-Fc:ALK3-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:115的氨基酸序列。

[0668] 该ALK3-Fc融合蛋白由下列核酸(SEQ ID NO:116)编码。

[0669]
```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51  AGTCTTCGTT TCGCCCGGCG CCCAGAATCT GGATAGTATG CTTCATGGCA
101  CTGGGATGAA ATCAGACTCC GACCAGAAAA AGTCAGAAAA TGGAGTAACC
151  TTAGCACCAG AGGATACCTT GCCTTTTTTA AAGTGCTATT GCTCAGGGCA
```

[0670]

```
 201  CTGTCCAGAT GATGCTATTA ATAACACATG CATAACTAAT GGACATTGCT
 251  TTGCCATCAT AGAAGAAGAT GACCAGGGAG AAACCACATT AGCTTCAGGG
 301  TGTATGAAAT ATGAAGGATC TGATTTTCAG TGCAAAGATT CTCCAAAAGC
 351  CCAGCTACGC CGGACAATAG AATGTTGTCG GACCAATTTA TGTAACCAGT
 401  ATTTGCAACC CACACTGCCC CCTGTTGTCA TAGGTCCGTT TTTTGATGGC
 451  AGCATTCGAA CCGGTGGTGG AACTCACACA TGCCCACCGT GCCCAGCACC
 501  TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
 551  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
 601  GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT
 651  GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA
 701  CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT
 751  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT
 801  CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
 851  ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
 901  ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA
 951  GAGCAATGGG CAGCCGGAGA ACAACTACGA CACCACGCCT CCCGTGCTGG
1001  ACTCCGACGG CTCCTTCTTC CTCTATAGCG ACCTCACCGT GGACAAGAGC
1051  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
1101  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGT
```

(SEQ ID NO: 116)

[0671] 成熟的ALK3-Fc融合蛋白序列(SEQ ID NO:117)如下,可任选提供C端加入赖氨酸的序列。

[0672]

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

[0673] SEQ ID NO:102和SEQ ID NO:117的ActRIIB-Fc和ALK3-Fc融合蛋白分别可共表达并从CHO细胞系中纯化以产生包含ActRIIB-Fc:ALK3-Fc的异聚体复合体。

[0674] 在分别在SEQ ID NO:401-402和407-408的ActRIIB-Fc和ALK3-Fc多肽序列中说明的使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例1中论述了ActRIIB-Fc融合多肽序列。

[0675] ALK3-Fc融合多肽的互补形式(SEQ ID NO:407)如下:

[0676]

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

[0677] 前导序列和接头用下划线表示。为了引导用上文SEQ ID NO401和402的ActRIIB-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入上文双下划线所示ALK3融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:407的氨基酸序列。

[0678] 成熟的ALK3-Fc融合蛋白序列(SEQ ID NO:408)如下，并可任选提供从C端脱去赖氨酸(K)的序列。

[0679]

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

[0680] SEQ ID NO:402和SEQ ID NO:408的ActRIIB-Fc和ALK3-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIB-Fc:ALK3-Fc的异聚体复合体。

[0681] 不同ActRIIB-Fc:ALK3-Fc复合体可的纯化通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0682] 实施例5.与ActRIIB-Fc同二聚体和ALK3-Fc同二聚体相比ActRIIB-Fc:ALK3-Fc异二聚体的配体结合特征

[0683] 采用基于Biacore™的结合测定法以对上述ActRIIB-Fc:ALK3-Fc异二聚体复合体的配体结合选择性与ActRIIB-Fc和ALK3-Fc同二聚体复合体的配体结合选择性进行比较。使用抗Fc抗体将ActRIIB-Fc:ALK3-Fc异二聚体、ActRIIB-Fc同二聚体和ALK3-Fc同二聚体独立俘获在系统中。注入配体，并使之在俘获的受体蛋白上流过。结果概括于下表，其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0684]

与 ActRIIB-Fc 同二聚体和 ALK3-Fc 同二聚体相比 ActRIIB-Fc:ALK3-Fc 异二聚体的配体结合特征									
配体	ActRIIB-Fc 同二聚体			ALK3-Fc 同二聚体			ActRIIB-Fc:ALK3-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
激活素 A	1.3×10^7	1.4×10^{-4}	11	无结合			3.4×10^7	5.0×10^{-3}	150
激活素 B	5.1×10^6	1.0×10^{-4}	20	无结合			2.8×10^6	5.7×10^{-4}	200
BMP2	瞬时*		> 66000	6.8×10^5	8.9×10^{-5}	130	8.0×10^6	1.1×10^{-5}	1
BMP4	---			3.0×10^5	5.3×10^{-5}	180	2.6×10^6	6.5×10^{-6}	3
BMP5	2.6×10^7	7.5×10^{-2}	2900	2.9×10^4	2.0×10^{-3}	70000	9.0×10^5	5.8×10^{-4}	640
BMP6	3.5×10^7	6.8×10^{-3}	190	1.4×10^5	4.9×10^{-3}	35000	2.0×10^7	2.9×10^{-4}	15
BMP7	8.8×10^6	1.4×10^{-2}	1600	1.2×10^6	1.8×10^{-2}	15000	8.2×10^5	1.5×10^{-3}	1900
BMP9	3.9×10^7	1.3×10^{-3}	34	无结合			瞬时*		>33000
BMP10	5.9×10^7	2.0×10^{-4}	4	无结合			3.0×10^7	9.4×10^{-4}	31
GDF3	1.6×10^6	2.3×10^{-3}	1400	无结合			1.4×10^7	8.2×10^{-2}	5900
GDF5	瞬时*		> 9600	4.8×10^5	1.1×10^{-2}	22000	1.2×10^7	8.3×10^{-4}	70
GDF6	---			3.4×10^4	1.3×10^{-3}	40000	2.8×10^5	4.5×10^{-4}	1600
GDF7	瞬时*		> 12000	2.2×10^5	2.7×10^{-2}	12000	7.5×10^6	4.0×10^{-4}	52
GDF8	8.3×10^5	2.3×10^{-4}	280	无结合			3.0×10^6	9.2×10^{-4}	310

[0685]

GDF11	5.0×10^7	1.1×10^{-4}	2	无结合			1.6×10^7	1.1×10^{-3}	66
* 因相互作用的瞬时性质而不确定 --- 未测									

[0686] 这些比较结合数据表明相对于ActRIIB-Fc同二聚体或ALK3-Fc同二聚体，ActRIIB-Fc:ALK3-Fc异二聚体的结合特征/选择性改变。ActRIIB-Fc:ALK3-Fc异二聚体以异常高的亲和力结合BMP2和BMP4，并且显示与任一种同二聚体相比与BMP5、BMP6、BMP7、GDF5、GDF6和GDF7的结合大大增强。与ActRIIB同二聚体相比，ActRIIB-Fc:ALK3-Fc异二聚体显示与激活素A、激活素B、BMP10、GDF8和GDF11的结合减弱，并且还区分这些配体至较大程度，尤其激活素A和激活素B。另外，ActRIIB-Fc同二聚体以高亲和力结合BMP9和GDF3的能力对于ActRIIB-Fc:ALK3-Fc异二聚体不存在。参见图7。

[0687] 这些结果因此表明ActRIIB-Fc:ALK3-Fc异二聚体是激活素B、GDF5/GDF6/GDF7配体亚家族和几种关键BMP配体(最值得注意的BMP9除外)的选择性抑制剂。因此,在其中这类选择性拮抗作用是有利的某些应用中,ActRIIB-Fc:ALK3-Fc异二聚体将比ActRIIB-Fc同二聚体或ALK3-Fc同二聚体更有用。实例包括其中需要保持BMP2、BMP4、BMP5和BMP6或激活素B的拮抗作用但使具有肌肉合成作用的一种或多种配体(例如激活素A和GDF8)或具有血管生成作用的配体(例如BMP9和BMP10)的拮抗作用减到最小的治疗应用。

[0688] 实施例6.小鼠中与ActRIIB-Fc同二聚体和ALK3-Fc同二聚体相比ActRIIB-Fc:ALK3-Fc异二聚体的活性特征

[0689] 在小鼠中测试同二聚体和异二聚体复合体以研究其体内活性特征的差异。在10周龄(n=5只小鼠/周)时开始,腹膜内给予野生型C57BL/6小鼠ActRIIB-Fc同二聚体(10mg/kg)、ALK3-Fc同二聚体(10mg/kg)、ActRIIB-Fc:ALK4-Fc异二聚体(3或10mg/kg)或溶媒(磷酸缓冲盐水,PBS)每周两次达6.5周(46天)。研究终点包括在基线和研究完成(6.5周)通过核磁共振(NMR)测定的体重、总脂肪质量和在基线和6.5周通过双能X光吸光测定法(DEXA)测定的总骨矿物质密度。

[0690]

与溶媒相比野生型小鼠中 ActRIIB-Fc 和 ALK3-Fc 复合体的活性

终点 6.5 周	ActRIIB-Fc 同二聚体 10 mg/kg	ALK3-Fc 同二聚体 10 mg/kg	ActRIIB-Fc:ALK3-Fc 异二聚体	
			10 mg/kg	3 mg/kg
体重	↑23% *	↓3%	↓0.5%	↓1%
总脂肪质量	↓41% *	↓12%	↓14% *	↓18% *
总骨矿物质密度	↑8% *	↑6% *	↑9% *	↑10% *
*$P<0.05$ 相对于溶媒				

[0691] 研究结果概括于上表中。正如所预期的,所有与溶媒相比,ActRIIB-Fc同二聚体显著增加体重和总骨矿物质密度,并显著减少总脂肪质量。同样正如所预期的,与溶媒相比,ALK3-Fc同二聚体显著增加总骨矿物质密度,但与ActRIIB-Fc同二聚体不同,不显著改变体重或总脂肪质量。ActRIIB-Fc:ALK3-Fc异二聚体值得注意地显示不同于ActRIIB-Fc同二聚体或ALK3-Fc同二聚体的活性特征。小鼠用任一种剂量水平的ActRIIB-Fc:ALK4-Fc异二聚体治疗显著增加骨矿物质密度,至少与任一种同二聚体一样好。然而,与ALK3-Fc同二聚体不同,ActRIIB-Fc:ALK3-Fc异二聚体显著减少脂肪质量,且与ActRIIB-Fc同二聚体不一样,ActRIIB-Fc:ALK3-Fc异二聚体显著减少脂肪质量而不改变体重。因此,ActRIIB-Fc:ALK3-Fc异二聚体对骨发挥有益作用,并且对脂肪组织发挥可能的有益作用。这种新的选择性在例如治疗需要对骨的刺激作用和对脂肪的抑制作用但不需要改变体重的患者中将是有用的。

[0692] 实施例7.ActRIIB-Fc:ALK7-Fc异二聚体的产生

[0693] 申请人构建了包含人ActRIIB和人ALK7的胞外域(其各自与具有位于胞外域和Fc

结构域之间的接头的Fc结构域融合)的可溶性ActRIIB-Fc:ALK7-Fc异聚体复合体。各个构建体分别被称为ActRIIB-Fc和ALK7-Fc。

[0694] 通过类似于实施例1所述方法可引导异聚体ALK7-Fc:ActRIIB-Fc的形成。

[0695] 在第一种方法中,ActRIIB-Fc融合蛋白的多肽序列和其编码核酸序列在上文实施例1中以SEQ ID NO:100-102提供。

[0696] 互补ALK7-Fc融合蛋白使用TPA前导序列,并列举如下(SEQ ID NO:112):

[0697]

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251  EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
301  LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
     (SEQ ID NO: 112)
```

[0698] 信号序列和接头序列用下划线表示。为了促进ActRIIB-Fc:ALK7-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:112的氨基酸序列。

[0699] 该ALK7-Fc融合蛋白由下列核酸(SEQ ID NO:113)编码:

[0700]

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGGACTGAA GTGTGTATGT CTTTTGTGTG
 101  ATTCTTCAAA CTTTACCTGC CAAACAGAAG GAGCATGTTG GGCATCAGTC
 151  ATGCTAACCA ATGGAAAAGA GCAGGTGATC AAATCCTGTG TCTCCCTTCC
 201  AGAACTGAAT GCTCAAGTCT TCTGTCATAG TTCCAACAAT GTTACCAAAA
 251  CCGAATGCTG CTTCACAGAT TTTTGCAACA ACATAACACT GCACCTTCCA
 301  ACAGCATCAC CAAATGCCCC AAAACTTGGA CCCATGGAGA CCGGTGGTGG
 351  AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG GGGGGACCGT
 401  CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG
 451  ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA
 501  GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA
 551  CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC
 601  CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA
 651  GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG
 701  CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG
```

[0701]

751 GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT
801 CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA
851 ACAACTACGA CACCACGCCT CCCGTGCTGG ACTCCGACGG CTCCTTCTTC
901 CTCTATAGCG ACCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT
951 CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA
1001 AGAGCCTCTC CCTGTCTCCG GGT (SEQ ID NO: 113)

[0702] 预期成熟的ALK7-Fc融合蛋白序列(SEQ ID NO:114)如下,并可任选提供C端加入赖氨酸的序列。

[0703]

1 GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
51 CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNG关键KCKVSNKALP
201 APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
251 EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ GNVFSCSVMH
301 EALHNHYTQK SLSLSPG (SEQ ID NO: 114)

[0704] SEQ ID NO:102和SEQ ID NO:114的ActRIIB-Fc和ALK7-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIB-Fc:ALK7-Fc的异聚体复合体。

[0705] 在分别在SEQ ID NO:401-402和405-406的ActRIIB-Fc和ALK7-Fc多肽序列举例说明的使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例1中论述了ActRIIB-Fc融合多肽序列。

[0706] ALK7-Fc融合多肽的互补形式(SEQ ID NO:405)如下:

[0707]

1 MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
51 MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101 TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR
251 EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301 LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
(SEQ ID NO: 405)

[0708] 前导序列和接头序列用下划线表示。为了引导用上文SEQ ID NO401和402的ActRIIB-Fc融合多肽形成异二聚体,可将4个氨基酸取代引入上文双下划线所示ALK7融合多肽的Fc结构域中。此外,Fc结构域的C端赖氨酸残基可缺失。可任选提供从C端脱去赖氨酸的SEQ ID NO:405的氨基酸序列。

[0709] 预期成熟的ALK7-Fc融合蛋白序列(SEQ ID NO:406)如下,并可任选提供从C端脱去赖氨酸的序列。

1 GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF

51 CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC

101 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV

[0710] 151 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNG关键KCKVSNKALP

201 APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV KGFYPSDIAV

251 EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH

301 EALHNHYTQK SLSLSPGK (SEQ ID NO: 406)

[0711] SEQ ID NO:402和SEQ ID NO:406的ActRIIB-Fc和ALK7-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含的异聚体复合体ActRIIB-Fc:ALK7-Fc。

[0712] 不同ActRIIB-Fc:ALK7-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0713] 实施例8.与ActRIIB-Fc同二聚体和ALK7-Fc同二聚体相比ActRIIB-Fc:ALK7-Fc异二聚体的配体结合特征

[0714] 采用基于Biacore™的结合测定法以对上文所述ActRIIB-Fc:ALK7-Fc异二聚体复合体的配体结合选择性与ActRIIB-Fc和ALK7-Fc同二聚体复合体的进行比较。使用抗Fc抗体将ActRIIB-Fc:ALK7-Fc异二聚体、ActRIIB-Fc同二聚体和ALK7-Fc同二聚体独立俘获在系统中。注入配体,并使之在俘获的受体蛋白上流过。结果概括于下表,其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0715]

与 ActRIIB-Fc 同二聚体和 ALK7-Fc 同二聚体相比 ActRIIB-Fc:ALK7-Fc 异二聚体的配体结合特征

[0716]

配体	ActRIIB-Fc 同二聚体			ALK7-Fc 同二聚体			ActRIIB-Fc:ALK7-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
激活素 A	1.3×10^7	1.4×10^{-4}	11	无结合			4.4×10^7	1.9×10^{-3}	43
激活素 B	1.5×10^7	1.6×10^{-4}	8	无结合			1.2×10^7	2.0×10^{-4}	17
BMP5	2.6×10^7	7.5×10^{-2}	2900	无结合			1.5×10^5	8.5×10^{-3}	57000
BMP6	2.4×10^7	3.9×10^{-3}	160	无结合			1.2×10^6	6.3×10^{-3}	5300
BMP9	1.2×10^8	1.2×10^{-3}	10	无结合			瞬时*		>1400
BMP10	5.9×10^6	1.5×10^{-4}	25	无结合			1.5×10^7	2.8×10^{-3}	190
GDF3	1.4×10^6	2.2×10^{-3}	1500	无结合			2.3×10^6	1.0×10^{-2}	4500
GDF8	3.5×10^6	2.4×10^{-4}	69	无结合			3.7×10^6	1.0×10^{-3}	270
GDF11	9.6×10^7	1.5×10^{-4}	2	无结合			9.5×10^7	7.5×10^{-4}	8
* 因相互作用的瞬时性质而不确定									

[0717] 这些比较结合数据表明与ActRIIB-Fc同二聚体或ALK7-Fc同二聚体相比，ActRIIB-Fc:ALK7-Fc异二聚体具有不同的结合特征。引人关注的是，与ActRIIB-Fc同二聚体牢固结合的4-5种配体(激活素A、BMP10、GDF8和GDF11)显示与ActRIIB-Fc:ALK7-Fc异二聚体的结合减弱，例外的是保持与异二聚体紧密结合的激活素B。另外，与ActRIIB-Fc同二聚体中等结合的3种配体(GDF3、BMP6和尤其BMP9)显示与ActRIIB-Fc:ALK7-Fc异二聚体的结合减弱。相比之下，BMP5以中等强度结合ActRIIB-Fc:ALK7异二聚体但只与ActRIIB-Fc同二聚体弱结合。无受测配体与ALK7-Fc同二聚体结合。参见图8。

[0718] 这些结果因此表明与ActRIIB-Fc同二聚体相比，ActRIIB-Fc:ALK7-Fc异二聚体是激活素B的更高选择性拮抗剂。因此，在其中这类选择性拮抗作用是有利的某些应用中，ActRIIB-Fc:ALK7-Fc异二聚体将比ActRIIB-Fc同二聚体更有用。实例包括其中需要保持激活素B的拮抗作用但使激活素A、GDF3、GDF8、GDF11、BMP9或BMP10的一种或多种的拮抗作用减到最小的治疗应用。

[0719] 实施例9.ActRIIB-Fc:ALK2-Fc异二聚体的产生

[0720] 申请人构建了包含人ActRIIB和人ALK2的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIB-Fc:ALK2-Fc异聚体复合体。各个构建体分别被称为ActRIIB-Fc和ALK2-Fc。

[0721] 通过类似于实施例1所述方法可引导异聚体ActRIIB-Fc:ALK2-Fc的形成。

[0722] 在第一种方法中,实施例1中以SEQ ID NO:100-102提供ActRIIB-Fc融合蛋白的多肽序列和其编码核酸序列。

[0723] 互补ALK2-Fc融合蛋白使用TPA前导序列,并列举如下(SEQ ID NO:136):

[0724]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
301  DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPG      (SEQ ID NO: 136)
```

[0725] 信号序列和接头序列用下划线表示。为了促进ActRIIB-Fc:ALK2-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:136的氨基酸序列。

[0726] 该ALK2-Fc融合蛋白由下列核酸(SEQ ID NO:137)编码:

[0727]

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCATGGAAGA TGAGAAGCCC AAGGTCAACC
 101  CCAAACTCTA CATGTGTGTG TGTGAAGGTC TCTCCTGCGG TAATGAGGAC
 151  CACTGTGAAG GCCAGCAGTG CTTTTCCTCA CTGAGCATCA ACGATGGCTT
 201  CCACGTCTAC CAGAAAGGCT GCTTCCAGGT TTATGAGCAG GGAAAGATGA
 251  CCTGTAAGAC CCCGCCGTCC CCTGGCCAAG CTGTGGAGTG CTGCCAAGGG
 301  GACTGGTGTA ACAGGAACAT CACGGCCCAG CTGCCCACTA AAGGAAAATC
 351  CTTCCCTGGA ACACAGAATT TCCACTTGGA GACCGGTGGT GGAACTCACA
 401  CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
 451  CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
 501  GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
 551  TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
 601  CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
 651  CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA
 701  ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
 751  CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGAGGAGAT
 801  GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA
 851  GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC
 901  GACACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTATAG
 951  CGACCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001  GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
1051  TCCCTGTCTC CGGGT          (SEQ ID NO: 137)
```

[0728] 成熟的ALK2-Fc融合蛋白序列(SEQ ID NO:138)如下,并可任选提供C端加入赖氨酸的序列。

[0729]

```
   1  MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
  51  FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
 101  HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
 251  TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF LYSDLTVDKS
 301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G    (SEQ ID NO: 138)
```

[0730] SEQ ID NO:102和SEQ ID NO:138的ActRIIB-Fc和ALK2-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIB-Fc:ALK2-Fc的异聚体复合体。

[0731] 在使用分别以SEQ ID NO:401-402和421-422的ActRIIB-Fc和ALK2-Fc多肽序列举例说明的不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例1中论述了ActRIIB-Fc融合多肽序列。

[0732] ALK2-Fc融合多肽的互补形式(SEQ ID NO:421)如下:

[0733]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51   HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101   DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151   LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201   REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251   QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY
301   KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351   SLSPGK        (SEQ ID NO: 421)
```

[0734] 前导序列和接头序列用下划线表示。为了引导用上文SEQ ID NO401和402的ActRIIB-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入上文双下划线所示ALK2融合多肽的Fc结构域中。此外，Fc结构域的C端赖氨酸残基可缺失。可任选提供从C端脱去赖氨酸的SEQ ID NO:421的氨基酸序列。

[0735] 成熟的ALK2-Fc融合蛋白序列(SEQ ID NO:422)如下，并可任选提供从C端脱去赖氨酸的序列。

[0736]

```
  1   MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
 51   FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
101   HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151   VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201   GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
251   SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS
301   RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 422)
```

[0737] SEQ ID NO:402和SEQ ID NO:422的ActRIIB-Fc和ALK2-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIB-Fc:ALK2-Fc的异聚体复合体。

[0738] 不同ActRIIB-Fc:ALK2-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0739] 实施例10.与ActRIIB-Fc同二聚体和ALK2-Fc同二聚体相比ActRIIB-Fc:ALK2-Fc异二聚体的配体结合特征

[0740] 采用基于Biacore™的结合测定法以对上文所述ActRIIB-Fc:ALK2-Fc异二聚体复合体的配体结合选择性与ActRIIB-Fc和ALK2-Fc同二聚体复合体的进行比较。使用抗Fc抗体将ActRIIB-Fc:ALK2-Fc异二聚体、ActRIIB-Fc同二聚体和ALK2-Fc同二聚体独立俘获在系统中。注入配体，并使之在俘获的受体蛋白上流过。结果概括于下表，其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0741]

与 ActRIIB-Fc 同二聚体和 ALK2-Fc 同二聚体相比 ActRIIB-Fc:ALK2-Fc 异二聚体的配体结合特征									
配体	ActRIIB-Fc 同二聚体			ALK2-Fc 同二聚体			ActRIIB-Fc:ALK2-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
激活素 A	1.2×10^7	1.7×10^{-4}	15	无结合			3.4×10^7	2.6×10^{-3}	76
激活素 B	3.8×10^6	1.1×10^{-4}	28	无结合			3.2×10^6	1.5×10^{-4}	47
BMP5	3.8×10^6	3.7×10^{-2}	9700	无结合			1.2×10^6	1.4×10^{-3}	1200
BMP7	8.8×10^6	1.4×10^{-2}	1600	无结合			1.5×10^7	2.6×10^{-3}	170
BMP9	3.9×10^7	1.3×10^{-3}	34	无结合			3.2×10^6	8.9×10^{-4}	280
BMP10	5.4×10^7	2.8×10^{-4}	5	无结合			5.5×10^7	2.9×10^{-3}	53
GDF3	1.2×10^6	2.0×10^{-3}	1700	无结合			1.8×10^6	1.2×10^{-2}	6500
GDF5	1.2×10^6	1.4×10^{-3}	1100	无结合			8.8×10^5	4.4×10^{-3}	5000
GDF6	1.5×10^5	5.7×10^{-3}	39000	无结合			瞬时*		> 240000
GDF8	2.5×10^6	3.2×10^{-4}	130	无结合			2.1×10^6	7.3×10^{-4}	360
GDF11	2.0×10^6	2.2×10^{-4}	110	无结合			1.6×10^6	9.3×10^{-4}	600
* 因相互作用的瞬时性质而不确定									

[0742] 这些比较结合数据表明ActRIIB-Fc:ALK2-Fc异二聚体显示不同于ActRIIB-Fc同二聚体或ALK2-Fc同二聚体的配体结合特征。ActRIIB-Fc:ALK2-Fc异二聚体显示与激活素B优先地牢固结合，因此类似于ActRIIB-Fc:ALK7-Fc异二聚体(参见实施例8)。然而，ActRIIB-Fc:ALK2-Fc异二聚体通过保持ActRIIB-Fc同二聚体特有的与BMP9紧密结合而部分不同于ActRIIB-Fc:ALK7-Fc，而ActRIIB-Fc:ALK7-Fc极弱地结合BMP9，如果多少有的话。无受测配体与ALK2-Fc同二聚体结合。参见图9。

[0743] 这些结果表明与ActRIIB-Fc同二聚体相比ActRIIB-Fc:ALK2-Fc异二聚体是激活素B的更高选择性拮抗剂。因此，ActRIIB-Fc:ALK2-Fc异二聚体在其中这类选择性拮抗作用是有利的某些应用中将是有用的。实例包括其中需要保持主要是激活素B的拮抗作用并补充对BMP9、GDF8和GDF11次要的拮抗作用的治疗应用。

[0744] 实施例11.ActRIIB-Fc:ALK5-Fc异二聚体的产生

[0745] 申请人构建了包含人ActRIIB和人ALK5的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIB-Fc:ALK5-Fc异聚体复合体。各个构建体分别被称为ActRIIB-Fc和ALK5-Fc。

[0746] 通过类似于实施例1所述方法可引导异聚体ActRIIB-Fc:ALK5-Fc的形成。

[0747] 在第一种方法中,实施例1中以SEQ ID NO:100-102提供ActRIIB-Fc融合蛋白的多肽序列和其编码核酸序列。

[0748] 互补ALK5-Fc融合蛋白使用TPA前导序列,并列举如下(SEQ ID NO:139):

[0749]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
 51  GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
101  NQDHCNKIEL PTTVKSSPGL GPVETGGGTH TCPPCPAPEL LGGPSVFLFP
151  PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
201  QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
251  EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYDTT
301  PPVLDSDGSF FLYSDLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
```

[0750]
```
351  PG    (SEQ ID NO: 139)
```

[0751] 信号序列和接头序列用下划线表示。为了促进ActRIIB-Fc:ALK5-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:139的氨基酸序列。

[0752] 该ALK5-Fc融合蛋白由下列核酸(SEQ ID NO:140)编码:

[0753]
```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGCGCTGCT CCCGGGGGCG ACGGCGTTAC
 101  AGTGTTTCTG CCACCTCTGT ACAAAAGACA ATTTTACTTG TGTGACAGAT
 151  GGGCTCTGCT TTGTCTCTGT CACAGAGACC ACAGACAAAG TTATACACAA
 201  CAGCATGTGT ATAGCTGAAA TTGACTTAAT TCCTCGAGAT AGGCCGTTTG
 251  TATGTGCACC CTCTTCAAAA ACTGGGTCTG TGACTACAAC ATATTGCTGC
 301  AATCAGGACC ATTGCAATAA AATAGAACTT CCAACTACTG TAAAGTCATC
 351  ACCTGGCCTT GGTCCTGTGG AAACCGGTGG TGGAACTCAC ACATGCCCAC
 401  CGTGCCCAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC
 451  CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG
 501  CGTGGTGGTG GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT
 551  ACGTGGACGG CGTGGAGGTG CATAATGCCA AGACAAAGCC GCGGGAGGAG
 601  CAGTACAACA GCACGTACCG TGTGGTCAGC GTCCTCACCG TCCTGCACCA
 651  GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC AACAAAGCCC
 701  TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
 751  GAACCACAGG TGTACACCCT GCCCCCATCC CGGGAGGAGA TGACCAAGAA
 801  CCAGGTCAGC CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG
 851  CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG AGAACAACTA CGACACCACG
 901  CCTCCCGTGC TGGACTCCGA CGGCTCCTTC TTCCTCTATA GCGACCTCAC
 951  CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA
1001  TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
1051  CCGGGT    (SEQ ID NO: 140)
```

[0754] 成熟的ALK5-Fc融合蛋白序列(SEQ ID NO:141)如下,并可任选提供C端加入赖氨

酸的序列。

[0755]
```
  1   ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
 51   DLIPRDRPFV CAPSSKTGSV TTTYCCNQDH CNKIELPTTV KSSPGLGPVE
101   TGGGTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
151   EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
```

[0756]
```
201   YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL
251   VKGFYPSDIA VEWESNGQPE NNYDTTPPVL DSDGSFFLYS DLTVDKSRWQ
301   QGNVFSCSVM HEALHNHYTQ KSLSLSPG   (SEQ ID NO: 141)
```

[0757] SEQ ID NO:102和SEQ ID NO:141的ActRIIB-Fc和ALK5-Fc融合蛋白分别可共表达并从CHO细胞系中纯化以产生包含ActRIIB-Fc:ALK5-Fc的异聚体复合体。

[0758] 在SEQ ID NO:401-402和423-424的ActRIIB-Fc和ALK5-Fc多肽序列中分别举例说明的使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例1中论述了ActRIIB-Fc融合多肽序列。

[0759] ALK5-Fc融合多肽的互补形式(SEQ ID NO:423)如下:

[0760]
```
  1   MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
 51   GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
101   NQDHCNKIEL PTTVKSSPGL GPVETGGGTH TCPPCPAPEL LGGPSVFLFP
151   PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
201   QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
251   EPQVCTLPPS REEMTKNQVS LSCAVKGFYP SDIAVEWESN GQPENNYKTT
301   PPVLDSDGSF FLVSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
351   PGK       (SEQ ID NO: 423)
```

[0761] 前导序列和接头序列用下划线表示。为了引导用上文SEQ ID NO401和402的ActRIIB-Fc融合多肽形成异二聚体,可将4个氨基酸取代引入上文双下划线所示ALK5融合多肽的Fc结构域中。此外,Fc结构域的C端赖氨酸残基可缺失。可任选提供从C端脱去赖氨酸的SEQ ID NO:423的氨基酸序列。

[0762] 成熟的ALK5-Fc融合蛋白序列(SEQ ID NO:424)如下,并可任选提供从C端脱去赖氨酸的序列。

[0763]
```
  1   ALLPGATALQ CFCHLCTKDN FTCVTDGLCF VSVTETTDKV IHNSMCIAEI
 51   DLIPRDRPFV CAPSSKTGSV TTTYCCNQDH CNKIELPTTV KSSPGLGPVE
101   TGGGTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
151   EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE
201   YKCKVSNKAL PAPIEKTISK AKGQPREPQV CTLPPSREEM TKNQVSLSCA
251   VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLVS KLTVDKSRWQ
```

[0764]
```
301   QGNVFSCSVM HEALHNHYTQ KSLSLSPGK      (SEQ ID NO: 424)
```

[0765] SEQ ID NO:402和SEQ ID NO:424的ActRIIB-Fc和ALK5-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIB-Fc:ALK5-Fc的异聚体复合体。

[0766] 不同ActRIIB-Fc:ALK5-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析

法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0767] 实施例12.与ActRIIB-Fc同二聚体和ALK5-Fc同二聚体相比ActRIIB-Fc:ALK5-Fc异二聚体的配体结合特征

[0768] 采用基于Biacore™的结合测定法以对上文所述ActRIIB-Fc:ALK5-Fc异二聚体复合体的配体结合选择性与ActRIIB-Fc和ALK5-Fc同二聚体复合体的进行比较。使用抗Fc抗体将ActRIIB-Fc:ALK5-Fc异二聚体、ActRIIB-Fc同二聚体和ALK5-Fc同二聚体独立俘获在系统中。注入配体，并使之在俘获的受体蛋白上流过。结果概括于下表，其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0769]

与 ActRIIB-Fc 同二聚体和 ALK5-Fc 同二聚体相比 ActRIIB-Fc:ALK5-Fc 异二聚体的配体结合特征									
配体	ActRIIB-Fc 同二聚体			ALK5-Fc 同二聚体			ActRIIB-Fc:ALK5-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
激活素 A	1.2×10^7	2.3×10^{-4}	19	无结合			3.6×10^7	1.6×10^{-3}	46
激活素 B	5.1×10^6	1.0×10^{-4}	20	无结合			3.9×10^6	3.1×10^{-4}	79
BMP6	6.4×10^6	7.0×10^{-3}	1100	无结合			9.3×10^6	1.5×10^{-2}	1700
BMP9	3.9×10^7	1.3×10^{-3}	34	无结合			瞬时*		> 6600
BMP10	2.1×10^7	3.8×10^{-4}	18	无结合			2.3×10^7	2.2×10^{-3}	150
GDF3	4.7×10^5	1.8×10^{-3}	3900	无结合			1.1×10^5	9.7×10^{-3}	8500
GDF8	1.2×10^6	1.9×10^{-4}	160	无结合			1.1×10^6	5.2×10^{-4}	490

[0770]

GDF11	1.9×10^6	1.4×10^{-4}	74	无结合			2.3×10^6	4.6×10^{-4}	600
* 因相互作用的瞬时性质而不确定									

[0771] 实施例13.ActRIIB-Fc:ALK6-Fc异二聚体的产生

[0772] 可产生包含各自可与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合的人ActRIIB和人ALK6的胞外域的可溶性ActRIIB-Fc:ALK6-Fc异聚体复合体。各个构建体分别被称为ActRIIB-Fc和ALK6-Fc。

[0773] 通过类似于实施例1所述方法可引导异聚体ActRIIB-Fc:ALK6-Fc的形成。

[0774] 在第一种方法中，在上文实施例1中以SEQ ID NO:100-102提供ActRIIB-Fc融合蛋白的多肽序列和其编码核酸序列。

[0775] 互补ALK6-Fc融合蛋白使用TPA前导序列，并列举如下(SEQ ID NO:142)：

[0776]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51   EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLESGDF QCRDTPIPHQ
101   RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151   PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201   VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251   IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW
301   ESNGQPENNY DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA
351   LHNHYTQKSL SLSPG      (SEQ ID NO: 142)
```

[0777] 信号序列和接头序列用下划线表示。为了促进ActRIIB-Fc:ALK6-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:142的氨基酸序列。

[0778] 该ALK6-Fc融合蛋白由下列核酸(SEQ ID NO:143)编码:

[0779]

```
  1   ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51   AGTCTTCGTT TCGCCCGGCG CCAAGAAAGA GGATGGTGAG AGTACAGCCC
101   CCACCCCCCG TCCAAAGGTC TTGCGTTGTA AATGCCACCA CCATTGTCCA
151   GAAGACTCAG TCAACAATAT TTGCAGCACA GACGGATATT GTTTCACGAT
201   GATAGAAGAG GATGACTCTG GGTTGCCTGT GGTCACTTCT GGTTGCCTAG
251   GACTAGAAGG CTCAGATTTT CAGTGTCGGG ACACTCCCAT TCCTCATCAA
301   AGAAGATCAA TTGAATGCTG CACAGAAAGG AACGAATGTA ATAAAGACCT
351   ACACCCTACA CTGCCTCCAT TGAAAAACAG AGATTTTGTT GATGGACCTA
401   TACACCACAG GACCGGTGGT GGAACTCACA CATGCCCACC GTGCCCAGCA
451   CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA
501   GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG
551   ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC
601   GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG
651   CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA
701   ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC
751   ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT
801   GTACACCCTG CCCCCATCCC GGGAGGAGAT GACCAAGAAC CAGGTCAGCC
851   TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
901   GAGAGCAATG GGCAGCCGGA GAACAACTAC GACACCACGC CTCCCGTGCT
951   GGACTCCGAC GGCTCCTTCT TCCTCTATAG CGACCTCACC GTGGACAAGA
1001  GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT
1051  CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGT
      (SEQ ID NO: 143)
```

[0781] 成熟的ALK6-Fc融合蛋白序列(SEQ ID NO:144)如下,并可任选提供C端加入赖氨酸的序列。

[0782]

```
  1    KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
 51    LPVVTSGCLG LESGDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101    KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151    TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201    LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251    EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
301    LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
       (SEQ ID NO: 144)
```

[0783] SEQ ID NO:102和SEQ ID NO:144的ActRIIB-Fc和ALK6-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIB-Fc:ALK6-Fc的异聚体复合体。

[0784] 在分别在SEQ ID NO:401-402和425-426ActRIIB-Fc和ALK6-Fc多肽序列举例说明的使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,可改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例1中论述了。ActRIIB-Fc融合多肽序列

[0785] ALK6-Fc融合多肽的互补形式(SEQ ID NO:425)如下:

[0786]

```
  1    MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51    EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLESGDF QCRDTPIPHQ
101    RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151    PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201    VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251    IEKTISKAKG QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW
301    ESNGQPENNY KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA
351    LHNHYTQKSL SLSPGK          (SEQ ID NO: 425)
```

[0787] 前导序列和接头序列用下划线表示。为了引导用上文SEQ ID NO401和402的ActRIIB-Fc融合多肽形成异二聚体,可将4个氨基酸取代引入上文双下划线所示ALK6融合多肽的Fc结构域中。此外,Fc结构域的C端赖氨酸残基可缺失。可任选提供从C端脱去赖氨酸的SEQ ID NO:425的氨基酸序列。

[0788] 成熟的ALK6-Fc融合蛋白序列(SEQ ID NO:426)可如下,并可任选提供从C端脱去赖氨酸的序列。

[0789]

```
  1    KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
 51    LPVVTSGCLG LESGDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101    KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151    TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201    LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR
251    EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301    LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
       (SEQ ID NO: 426)
```

[0790] SEQ ID NO:402和SEQ ID NO:426的ActRIIB-Fc和ALK6-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIB-Fc:ALK6-Fc的异聚体复合体。

[0791] 不同ActRIIB-Fc:ALK6-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析

法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0792] 实施例14.ActRIIA-Fc:ALK4-Fc异二聚体的产生

[0793] 申请人构建了包含人ActRIIA和人ALK4的胞外域(其各自分别与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIA-Fc:ALK4-Fc异聚体复合体。各个构建体分别被称为ActRIIA-Fc融合多肽和ALK4-Fc融合多肽。

[0794] 通过类似于实施例1所述方法可引导异聚体ActRIIA-Fc:ALK4-Fc的形成。在第一种方法中,改变一个Fc结构域以在相互作用面上引入阳离子氨基酸,同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。

[0795] ActRIIA-Fc多肽序列(SEQ ID NO:118)见下:

[0796]

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51  GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101  DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151  CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201  VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251  PAPIEKTISK AKGQPREPQV YTLPPSRKEM TKNQVSLTCL VKGFYPSDIA
301  VEWESNGQPE NNYKTTPPVL KSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
351  HEALHNHYTQ KSLSLSPGK            (SEQ ID NO: 118)
```

[0797] 前导序列和接头序列用下划线表示。为了促进ActRIIA-Fc:ALK4-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示ActRIIA融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:118的氨基酸序列。

[0798] 该ActRIIA-Fc融合蛋白由下列核酸序列(SEQ ID NO:119)编码:

[0799]

```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51  AGTCTTCGTT TCGCCCGGCG CCGCTATACT TGGTAGATCA GAAACTCAGG
101  AGTGTCTTTT CTTTAATGCT AATTGGGAAA AAGACAGAAC CAATCAAACT
151  GGTGTTGAAC CGTGTTATGG TGACAAAGAT AAACGGCGGC ATTGTTTTGC
201  TACCTGGAAG AATATTTCTG GTTCCATTGA AATAGTGAAA CAAGGTTGTT
251  GGCTGGATGA TATCAACTGC TATGACAGGA CTGATTGTGT AGAAAAAAAA
301  GACAGCCCTG AAGTATATTT CTGTTGCTGT GAGGGCAATA TGTGTAATGA
351  AAAGTTTTCT TATTTTCCGG AGATGGAAGT CACACAGCCC ACTTCAAATC
401  CAGTTACACC TAAGCCACCC ACCGGTGGTG GAACTCACAC ATGCCCACCG
451  TGCCCAGCAC CTGAACTCCT GGGGGGACCG TCAGTCTTCC TCTTCCCCCC
501  AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACATGCG
```

[0800]

```
 551   TGGTGGTGGA CGTGAGCCAC GAAGACCCTG AGGTCAAGTT CAACTGGTAC
 601   GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCGC GGGAGGAGCA
 651   GTACAACAGC ACGTACCGTG TGGTCAGCGT CCTCACCGTC CTGCACCAGG
 701   ACTGGCTGAA TGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGCCCTC
 751   CCAGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAAAGGGC AGCCCCGAGA
 801   ACCACAGGTG TACACCCTGC CCCCATCCCG GAAGGAGATG ACCAAGAACC
 851   AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTATCCCAG CGACATCGCC
 901   GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACTACA AGACCACGCC
 951   TCCCGTGCTG AAGTCCGACG GCTCCTTCTT CCTCTATAGC AAGCTCACCG
1001   TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
1051   CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
1101   GGGTAAA                    (SEQ ID NO: 119)
```

[0801] 成熟的ActRIIA-Fc融合多肽(SEQ ID NO:120)如下,并可任选提供从C端脱去赖氨酸的序列。

[0802]

```
  1   ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51   IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101   EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151   SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201   SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
251   SRKEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLKSDGS
301   FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
      (SEQ ID NO: 120)
```

[0803] 在此第一种方法中,在上文实施例1中以SEQ ID NO:104-106提供互补ALK4-Fc融合蛋白的多肽序列和其编码核酸序列。

[0804] SEQ ID NO:120和SEQ ID NO:106的ActRIIA-Fc和ALK4-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:ALK4-Fc的异聚体复合体。

[0805] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。

[0806] ActRIIA-Fc多肽序列(SEQ ID NO:409)见下:

[0807]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51   GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101   DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151   CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201   VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
```

[0808]

```
251   PAPIEKTISK AKGQPREPQV YTLPPCREEM TKNQVSLWCL VKGFYPSDIA
301   VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
351   HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 409)
```

[0809] 前导序列和接头序列用下划线表示。为了促进ActRIIA-Fc:ALK4-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并

将苏氨酸用色氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:409的氨基酸序列。

[0810] 成熟的ActRIIA-Fc融合多肽(SEQ ID NO:410)如下,并可任选提供从C端脱去赖氨酸的序列。

[0811]

```
  1  ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51  IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101  EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151  SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201  SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
251  CREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
301  FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
     (SEQ ID NO: 410)
```

[0812] 在此第二种方法中,在上文实施例1中以SEQ ID NO:403-404提供互补ALK4-Fc融合蛋白的多肽序列和其编码核酸序列。

[0813] SEQ ID NO:410和SEQ ID NO:404的ActRIIA-Fc和ALK4-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:ALK4-Fc的异聚体复合体。

[0814] 不同ActRIIA-Fc:ALK4-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0815] 实施例15.与ActRIIA-Fc同二聚体和ALK4-Fc同二聚体相比ActRIIA-Fc:ALK4-Fc异二聚体的配体结合特征

[0816] 采用基于Biacore™的结合测定法以对上文所述ActRIIA-Fc:ALK4-Fc异二聚体复合体的配体结合选择性与ActRIIA-Fc和ALK4-Fc同二聚体复合体的进行比较。使用抗Fc抗体将ActRIIA-Fc:ALK4-Fc异二聚体、ActRIIA-Fc同二聚体和ALK4-Fc同二聚体独立俘获在系统中。注入配体,并使之在俘获的受体蛋白上流过。结果概括于下表,其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0817]

与 ActRIIA-Fc 同二聚体和 ALK4-Fc 同二聚体相比 ActRIIA-Fc:ALK4-Fc 异二聚体的配体结合特征									
配体	ActRIIA-Fc 同二聚体			ALK4-Fc 同二聚体			ActRIIA-Fc:ALK4-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
激活素 A	1.4×10^7	6.2×10^{-4}	45	5.8×10^5	1.2×10^{-2}	20000	7.4×10^6	2.4×10^{-4}	32
激活素 B	1.1×10^7	1.1×10^{-4}	10	无结合			9.5×10^6	4.8×10^{-4}	50
激活素 AB	2.8×10^7	2.6×10^{-4}	9	1.8×10^6	3.6×10^{-3}	2000	1.8×10^7	2.3×10^{-4}	13
激活素 AC	2.2×10^7	7.9×10^{-3}	360	无结合			3.2×10^6	5.4×10^{-4}	170
BMP6	2.7×10^8	2.2×10^{-2}	800	无结合			5.4×10^6	1.2×10^{-2}	2200
BMP7	8.9×10^6	3.3×10^{-2}	3700	无结合			2.0×10^7	7.2×10^{-2}	3500
BMP9	瞬时*		>10000	---			无结合		
BMP10	2.9×10^7	2.5×10^{-3}	85	无结合			瞬时*		>6000
GDF3	1.5×10^6	3.6×10^{-3}	2400	---			4.9×10^7	4.8×10^{-3}	9800
GDF8	1.4×10^6	1.4×10^{-3}	99	1.3×10^5	1.9×10^{-3}	15000 †	1.8×10^7	2.8×10^{-3}	150
GDF11	7.3×10^7	9.2×10^{-4}	13	5.0×10^6	4.8×10^{-3}	970†	3.0×10^7	6.5×10^{-4}	22
* 因相互作用的瞬时性质而不确定 † 极低信号 --- 未测									

[0818] 这些比较结合数据表明相对于ActRIIA-Fc或ALK4-Fc同二聚体ActRIIA-Fc:ALK4-Fc异二聚体的结合特征/选择性改变。例如ActRIIA-Fc:ALK4-Fc异二聚体显示与ActRIIA-Fc同二聚体相比，与激活素A的结合增强，尤其与激活素AC的结合增强，同时保持与激活素AB和GDF11牢固结合。另外，具有对ActRIIA-Fc同二聚体有最高亲和力的配体激活素B显示对ActRIIA-Fc:ALK4-Fc异二聚体的亲和力降低(尽管仍在高亲和力范围内)。ActRIIA-Fc:ALK4-Fc异二聚体还显示与ActRIIA-Fc同二聚体相比与BMP10的结合显著减弱。参见图10。

[0819] 这些结果表明与ActRIIA-Fc同二聚体相比，ActRIIA-Fc:ALK4-Fc异二聚体是激活素A和激活素AB高于激活素B的更高选择性拮抗剂。另外，与ActRIIA-Fc同二聚体相比，ActRIIA-Fc:ALK4-Fc异二聚体对激活素AC的亲和力大大增强，对BMP10的亲和力则大大减弱。因此，在其中这类选择性拮抗作用是有利的某些应用中，ActRIIA-Fc:ALK4-Fc异二聚体将比ActRIIA-Fc同二聚体更有用。实例包括其中需要优先高于激活素B地拮抗激活素A和/或激活素AB，并达到对激活素AC的强抑制，同时避免BMP10的抑制的治疗应用。

[0820] 实施例16.BMPRII-Fc:ALK1-Fc异二聚体的产生

[0821] 申请人构建了包含人BMPRII和人ALK1的胞外域(其各自分别与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性BMPRII-Fc:ALK1-Fc异聚体复合体。各个构建体分别被称为BMPRII-Fc融合多肽和ALK1-Fc融合多肽,下面提供每个的序列。

[0822] 通过类似于实施例1所述方法可引导异聚体BMPRII-Fc:ALK1-Fc的形成。在第一种方法中,改变一个Fc结构域以在相互作用面上引入阳离子氨基酸,同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。

[0823] BMPRII-Fc多肽序列(SEQ ID NO:121)见下:

[0824]
```
  1   MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51   HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101   TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151   GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201   EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251   KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG
301   FYPSDIAVEW ESNGQPENNY KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN
351   VFSCSVMHEA LHNHYTQKSL SLSPGK      (SEQ ID NO: 121)
```

[0825]

[0826] 前导序列和接头序列用下划线表示。为了促进BMPRII-Fc:ALK1-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示BMPRII-Fc融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:121的氨基酸序列。

[0827] 此BMPRII-Fc融合蛋白由下列核酸序列(SEQ ID NO:122)编码:

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCGCAGAA TCAAGAACGC CTATGTGCGT
 101  TTAAAGATCC GTATCAGCAA GACCTTGGGA TAGGTGAGAG TAGAATCTCT
 151  CATGAAAATG GGACAATATT ATGCTCGAAA GGTAGCACCT GCTATGGCCT
 201  TTGGGAGAAA TCAAAAGGGG ACATAAATCT TGTAAAACAA GGATGTTGGT
 251  CTCACATTGG AGATCCCCAA GAGTGTCACT ATGAAGAATG TGTAGTAACT
 301  ACCACTCCTC CCTCAATTCA GAATGGAACA TACCGTTTCT GCTGTTGTAG
 351  CACAGATTTA TGTAATGTCA ACTTTACTGA GAATTTTCCA CCTCCTGACA
 401  CAACACCACT CAGTCCACCT CATTCATTTA ACCGAGATGA GACCGGTGGT
 451  GGAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC
 501  GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC
[0828]
 551  GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT
 601  GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA
 651  GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG
 701  TCCTCACCGT CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC
 751  AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA
 801  AGCCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC
 851  GGAAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
 901  TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA
 951  GAACAACTAC AAGACCACGC CTCCCGTGCT GAAGTCCGAC GGCTCCTTCT
1001  TCCTCTATAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
1051  GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA
1101  GAAGAGCCTC TCCCTGTCTC CGGGTAAA     (SEQ ID NO: 122)
```

[0829] 成熟的BMPRII-Fc融合多肽(SEQ ID NO:123)如下,并可任选提供从C端脱去赖氨酸的序列。

[0830]
```
   1  SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
  51  INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
 101  FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
 151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
```

[0831]
```
 201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
 251  PQVYTLPPSR KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
 301  PVLKSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
 351  GK        (SEQ ID NO: 123)
```

[0832] ALK1-Fc融合多肽的互补形式(SEQ ID NO:124)如下:

[0833]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
 51   GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
101   NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
151   KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201   YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251   PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP
301   PVLDSDGSFF LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351   G          (SEQ ID NO: 124)
```

[0834] 前导序列和接头序列用下划线表示。为了引导用上文SEQ ID NO:121和123的BMPRII-Fc融合多肽形成异二聚体,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示ALK1-Fc融合多肽的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:124的氨基酸序列。

[0835] 该ALK1-Fc融合蛋白由下列核酸(SEQ ID NO:125)编码:

[0836]

```
   1   ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51   AGTCTTCGTT TCGCCCGGCG CCGACCCTGT GAAGCCGTCT CGGGGCCCGC
 101   TGGTGACCTG CACGTGTGAG AGCCCACATT GCAAGGGGCC TACCTGCCGG
 151   GGGGCCTGGT GCACAGTAGT GCTGGTGCGG GAGGAGGGGA GGCACCCCCA
 201   GGAACATCGG GGCTGCGGGA ACTTGCACAG GGAGCTCTGC AGGGGCCGCC
 251   CCACCGAGTT CGTCAACCAC TACTGCTGCG ACAGCCACCT CTGCAACCAC
 301   AACGTGTCCC TGGTGCTGGA GGCCACCCAA CCTCCTTCGG AGCAGCCGGG
 351   AACAGATGGC CAGCTGGCCA CCGGTGGTGG AACTCACACA TGCCCACCGT
 401   GCCCAGCACC TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA
 451   AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT
 501   GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG
 551   TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG
 601   TACAACAGCA CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA
 651   CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC
 701   CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA
 751   CCACAGGTGT ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA
 801   GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG
 851   TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACGA CACCACGCCT
 901   CCCGTGCTGG ACTCCGACGG CTCCTTCTTC CTCTATAGCG ACCTCACCGT
 951   GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC
1001   ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
1051   GGT          (SEQ ID NO: 125)
```

[0837]

[0838] 成熟的ALK1-Fc融合蛋白序列(SEQ ID NO:126)如下,并可任选提供C端加入赖氨酸的序列。

[0839]

```
  1   DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
 51   LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
101   GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
151   DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
201   KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV
251   KGFYPSDIAV EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ
301   GNVFSCSVMH EALHNHYTQK SLSLSPG          (SEQ ID NO: 126)
```

[0840] SEQ ID NO:123和SEQ ID NO:126的BMPRII-Fc和ALK1-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含BMPRII-Fc:ALK1-Fc的异聚体复合体。

[0841] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。

[0842] BMPRII-Fc多肽序列(SEQ ID NO:411)见下:

[0843]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51   HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101   TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151   GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201   EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251   KVSNKALPAP IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG
301   FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
351   VFSCSVMHEA LHNHYTQKSL SLSPGK       (SEQ ID NO: 411)
```

[0844] 前导序列和接头序列用下划线表示。为了促进BMPRII-Fc:ALK1-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:411的氨基酸序列。

[0845] 成熟的BMPRII-Fc融合多肽(SEQ ID NO:412)如下,并可任选提供从C端脱去赖氨酸(K)的序列。

[0846]

```
  1   SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
 51   INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
101   FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
151   KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201   YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251   PQVYTLPPCR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP
301   PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351   GK       (SEQ ID NO: 412)
```

[0847] ALK1-Fc融合多肽的互补形式(SEQ ID NO:413)如下:

[0848]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
 51   GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
101   NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
151   KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201   YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251   PQVCTLPPSR EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP
301   PVLDSDGSFF LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351   GK       (SEQ ID NO: 413)
```

[0849] 前导序列和接头序列用下划线表示。为了引导用上文SEQ ID NO:411和412的BMPRII-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入上文双下划线所示ALK1融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:413的氨基酸序列。

[0850] 成熟的ALK1-Fc融合蛋白序列(SEQ ID NO:414)如下，并可任选提供从C端脱去赖氨酸的序列。

[0851]

```
  1   DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
 51   LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
101   GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
151   DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
201   KCKVSNKALP APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV
251   KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ
301   GNVFSCSVMH EALHNHYTQK SLSLSPGK       (SEQ ID NO: 414)
```

[0852] SEQ ID NO:412和SEQ ID NO:414的BMPRII-Fc和ALK1-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含BMPRII-Fc:ALK1-Fc的异聚体复合体。

[0853] 不同BMPRII-Fc:ALK1-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0854] 实施例17.与BMPRII-Fc同二聚体和ALK1-Fc同二聚体相比BMPRII-Fc:ALK1-Fc异二聚体的配体结合特征

[0855] 采用基于Biacore™的结合测定法以对上文所述BMPRII-Fc:ALK1-Fc异二聚体复合体的配体结合选择性与BMPRII-Fc和ALK1-Fc同二聚体复合体的进行比较。使用抗Fc抗体将BMPRII-Fc:ALK1-Fc异二聚体、BMPRII-Fc同二聚体和ALK1-Fc同二聚体独立俘获在系统中。注入配体，并使之在俘获的受体蛋白上流过。结果概括于下表，其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0856]

与 BMPRII-Fc 同二聚体和 ALK1-Fc 同二聚体相比 BMPRII-Fc:ALK1-Fc 异二聚体的配体结合特征									
配体	BMPRII-Fc 同二聚体			ALK1-Fc 同二聚体			BMPRII-Fc:ALK1-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
BMP9	1.2×10^7	2.6×10^{-2}	2100	7.8×10^6	1.3×10^{-4}	16	1.2×10^6	4.1×10^{-4}	360
BMP10	2.6×10^7	2.5×10^{-3}	100	4.1×10^6	1.6×10^{-4}	38	1.5×10^7	3.5×10^{-4}	23
BMP15	9.9×10^6	2.8×10^{-3}	290	无结合			1.2×10^7	4.2×10^{-2}	3500

[0857] 这些比较结合数据表明BMPRII-Fc:ALK1-Fc异二聚体具有不同于BMPRII-Fc同二聚体但与ALK1-Fc同二聚体的类似的结合特征/选择性。例如BMPRII-Fc:ALK1-Fc异二聚体大多保持ALK1-Fc同二聚体特有的与BMP9和BMP10牢固结合;然而,异二聚体显示用同二聚体不存在的针对BMP10优于BMP9的适度选择性。此外与ALK1-Fc同二聚体不一样,BMPRII-Fc:ALK1-Fc异二聚体与BMP15结合,尽管其亲和力比BMPRII-Fc同二聚体的弱大约一个数量级。参见图11。因此,BMPRII-Fc:ALK1-Fc异二聚体将意料不到地用于其中BMP9特别是BMP10的选择性拮抗作用对于例如抑制血管生成是有利的某些治疗应用,或用于其中BMP15的拮抗作用也是有利的应用中。

[0858] 实施例18.BMPRII-Fc:ALK2-Fc异二聚体的产生

[0859] 申请人构建了包含人BMPRII和人ALK2的胞外域(其各自分别与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性BMPRII-Fc:ALK2-Fc异聚体复合体。各个构建体分别被称为BMPRII-Fc融合多肽和ALK2-Fc融合多肽,本文提供各自的序列。

[0860] 通过类似于实施例1所述方法可引导异聚体BMPRII-Fc:ALK2-Fc的形成。在第一种方法中,改变一个Fc结构域以在相互作用面上引入阳离子氨基酸,同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。上文实施例1中以SEQ ID NO:121-123提供BMPRII-Fc融合多肽的多肽序列和其编码核酸序列。为了促进BMPRII-Fc:ALK2-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入实施例16所示BMPRII-Fc融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:121和123的氨基酸序列。

[0861] 实施例9中以SEQ ID NO:136-138提供互补ALK2-Fc融合多肽的多肽序列和其编码核酸序列。为了引导用SEQ ID NO:121和123的BMPRII-Fc融合多肽形成异二聚体,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入实施例9所示ALK2-Fc融合多肽的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:136和138的氨基酸序列。

[0862] SEQ ID NO:123和SEQ ID NO:138的BMPRII-Fc和ALK2-Fc融合多肽分别可共表达并从CHO细胞系中纯化,以产生包含BMPRII-Fc:ALK2-Fc的异聚体复合体。

[0863] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中论述了BMPRII-Fc融合多肽序列(SEQ ID NO:411-412)。为了促进BMPRII-Fc:ALK2-Fc异二聚体而非可能的同二聚

体复合体的任一个的形成，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入实施例16所示BMPRII-Fc多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:411和412的氨基酸序列。

[0864] 实施例9中论述了互补ALK2-Fc融合多肽的多肽序列(SEQ ID NO:421-422)。为了引导用SEQ ID NO:411-412的BMPRII-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入实施例9所示ALK2融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:421-422的氨基酸序列。

[0865] SEQ ID NO:412和SEQ ID NO:422的BMPRII-Fc和ALK2-Fc融合多肽分别可共表达并从CHO细胞系中纯化，以产生包含BMPRII-Fc:ALK2-Fc的异聚体复合体。

[0866] 不同BMPRII-Fc:ALK2-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0867] 实施例19.与BMPRII-Fc同二聚体和ALK2-Fc同二聚体相比BMPRII-Fc:ALK2-Fc异二聚体的配体结合特征

[0868] 采用基于Biacore™的结合测定法以对上文所述BMPRII-Fc:ALK2-Fc异二聚体复合体的配体结合选择性与BMPRII-Fc和ALK2-Fc同二聚体复合体的进行比较。使用抗Fc抗体将BMPRII-Fc:ALK2-Fc异二聚体、BMPRII-Fc同二聚体和ALK2-Fc同二聚体独立俘获在系统中。注入配体，并使之在俘获的受体蛋白上流过。结果概括于下表，其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0869]

与 BMPRII-Fc 同二聚体和 ALK2-Fc 同二聚体相比
BMPRII-Fc:ALK2-Fc 异二聚体的配体结合特征

[0870]

配体	BMPRII-Fc 同二聚体			ALK2-Fc 同二聚体			BMPRII-Fc:ALK2-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
激活素 B	1.9×10^6	4.9×10^{-3}	2600	无结合			5.9×10^5	3.1×10^{-3}	5200
BMP5	1.9×10^6	1.9×10^{-2}	9900	无结合			1.8×10^6	5.0×10^{-3}	2800
BMP7	瞬时*		> 93000	无结合			1.5×10^7	1.2×10^{-2}	760
BMP9	4.5×10^7	7.3×10^{-2}	1600	无结合			1.0×10^7	5.1×10^{-3}	500
BMP10	3.8×10^7	5.0×10^{-3}	130	无结合			1.1×10^8	3.4×10^{-2}	300
BMP15	5.8×10^6	4.2×10^{-3}	720	无结合			9.6×10^6	1.1×10^{-2}	1100
* 因相互作用的瞬时性质而不确定									

[0871] 实施例20.BMPRII-Fc:ALK3-Fc异二聚体的产生

[0872] 申请人构建了包含人BMPRII和人ALK3的胞外域(其各自分别与具有位于胞外域和

Fc结构域之间的接头的Fc结构域融合)的可溶性BMPRII-Fc:ALK3-Fc异聚体复合体。各个构建体分别被称为BMPRII-Fc融合多肽和ALK3-Fc融合多肽,本文提供各自的序列。

[0873] 通过类似于实施例1所述方法可引导异聚体BMPRII-Fc:ALK3-Fc的形成。在第一种方法中,改变一个Fc结构域以在相互作用面上引入阳离子氨基酸,同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。上文实施例1中以SEQ ID NO:121-123提供BMPRII-Fc融合多肽的多肽序列和其编码核酸序列。为了促进BMPRII-Fc:ALK3-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入实施例16所示BMPRII-Fc融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:121和123的氨基酸序列。

[0874] 在实施例4中以SEQ ID NO:115-117提供互补ALK3-Fc融合多肽的多肽序列和其编码核酸序列。为了引导用SEQ ID NO:121和123的BMPRII-Fc融合多肽形成异二聚体,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入实施例4所示ALK3-Fc融合多肽的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:115和117的氨基酸序列。

[0875] SEQ ID NO:123和SEQ ID NO:117的BMPRII-Fc和ALK3-Fc融合多肽分别可共表达并从CHO细胞系中纯化,以产生包含BMPRII-Fc:ALK3-Fc的异聚体复合体。

[0876] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中论述了BMPRII-Fc融合多肽序列(SEQ ID NO:411-412)。为了促进BMPRII-Fc:ALK3-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入实施例16所示BMPRII-Fc多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:411和412的氨基酸序列。

[0877] 实施例4中论述了互补ALK3-Fc融合多肽的多肽序列(SEQ ID NO:407-408)。为了引导用SEQ ID NO:411-412的BMPRII-Fc融合多肽形成异二聚体,可将4个氨基酸取代引实施例4所示ALK3融合多肽入的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:407和408的氨基酸序列。

[0878] SEQ ID NO:412和SEQ ID NO:408的BMPRII-Fc和ALK3-Fc融合多肽分别可共表达并从CHO细胞系中纯化,以产生包含BMPRII-Fc:ALK3-Fc的异聚体复合体。

[0879] 不同BMPRII-Fc:ALK3-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0880] 实施例21.与BMPRII-Fc同二聚体和ALK3-Fc同二聚体相比BMPRII-Fc:ALK3-Fc异二聚体的配体结合特征

[0881] 采用基于Biacore™的结合测定法以对上文所述BMPRII-Fc:ALK3-Fc异二聚体复合体的配体结合选择性与BMPRII-Fc和ALK3-Fc同二聚体复合体的进行比较。使用抗Fc抗体将BMPRII-Fc:ALK3-Fc异二聚体、BMPRII-Fc同二聚体和ALK3-Fc同二聚体独立俘获在系统中。注入配体,并使之在俘获的受体蛋白上流过。结果概括于下表,其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0882]

与 BMPRII-FC 同二聚体和 ALK3-FC 同二聚体相比 BMPRII-FC:ALK3-FC 异二聚体的配体结合特征									
配体	BMPRII-FC 同二聚体			ALK3-FC 同二聚体			BMPRII-FC:ALK3-FC 异二聚体		
	K_A (1/MS)	K_D (1/S)	K_D (PM)	K_A (1/MS)	K_D (1/S)	K_D (PM)	K_A (1/MS)	K_D (1/S)	K_D (PM)
激活素 B	$2.0 X10^7$	7.5 $X10^{-2}$	3800	无结合			MINIMAL 结合		
BMP2	瞬时*		>2 $X10^6$	$6.2 X10^5$	1.4 $X10^{-4}$	230	$2.9 X10^6$	1.5 $X10^{-4}$	51
BMP4	---			$2.6 X10^5$	5.5 $X10^{-5}$	210	$9.1 X10^5$	9.1 $X10^{-5}$	100
BMP5	---			$2.9 X10^4$	2.3 $X10^{-3}$	70000	$4.3 X10^5$	1.4 $X10^{-3}$	3200
BMP6	瞬时*		>8900	$1.4 X10^5$	4.9 $X10^{-3}$	35000	$3.6 X10^5$	5.9 $X10^{-4}$	1600
BMP7	瞬时*		>38000	$1.2 X10^6$	1.8 $X10^{-2}$	15000	$1.2 X10^7$	1.2 $X10^{-2}$	1000
BMP9	$1.2 X10^7$	2.6 $X10^{-2}$	2100	无结合			无结合		
BMP10	$2.6 X10^7$	2.5 $X10^{-3}$	100	---			$6.8 X10^5$	1.6 $X10^{-3}$	2400
BMP15	$9.9 X10^6$	2.8 $X10^{-3}$	290	---			$9.1 X10^5$	5.5 $X10^{-3}$	6000
GDF5	无结合			$4.3 X10^5$	1.1 $X10^{-2}$	22000	MINIMAL 结合		
GDF6	瞬时*		>88000	$3.4 X10^4$	1.3 $X10^{-3}$	40000	$1.4 X10^6$	1.9 $X10^{-3}$	1400
* 因相互作用的瞬时性质而不确定 --- 未测									

[0883] 这些比较结合数据表明BMPRII-Fc:ALK3-Fc异二聚体具有与BMPRII-Fc同二聚体显然不一样但也不同于ALK3-Fc同二聚体的配体结合选择性。BMPRII-Fc:ALK3-Fc异二聚体比ALK3-Fc同二聚体牢得多地与BMP6结合，反映在解离速率几乎为1/10。由于其与BMP2和BMP4的结合大多未改变，因此BMPRII-Fc:ALK3异二聚体可被视为BMP2、BMP4和BMP6的联合抑制剂。这种结合特征与ALK3-Fc同二聚体的截然不同，后者与BMP4和BMP2异常牢固地结合将其确定为与具有中等水平结合的4种配体(包括BMP6)相比对该配体对有高度选择性。参见图12。因此，BMPRII-Fc:ALK3-Fc异二聚体将意料不到地用于其中BMP2、BMP4和BMP6的联合拮抗作用是有利的某些治疗应用。

[0884] 实施例22.BMPRII-Fc:ALK4-Fc异二聚体的产生

[0885] 申请人构建了包含人BMPRII和人ALK4的胞外域(其各自分别与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性BMPRII-Fc:ALK4-Fc异聚体复合体。各个构建体分别被称为BMPRII-Fc融合多肽和ALK4-Fc融合多肽,本文提供各自的序列。

[0886] 通过类似于实施例1所述方法可引导异聚体BMPRII-Fc:ALK4-Fc的形成。在第一种方法中,改变一个Fc结构域以在相互作用面上引入阳离子氨基酸,同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。上文实施例1中以SEQ ID NO:121-123提供BMPRII-Fc融合多肽的多肽序列和其编码核酸序列。为了促进BMPRII-Fc:ALK4-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入实施例16所示BMPRII-Fc融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:121和123的氨基酸序列。

[0887] 实施例1中以SEQ ID NO:104-106提供互补ALK4-Fc融合多肽的多肽序列和其编码核酸序列。为了引导用SEQ ID NO:121和123的BMPRII-Fc融合多肽形成异二聚体,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入实施例1所示ALK4-Fc融合多肽的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:104和106的氨基酸序列。

[0888] SEQ ID NO:123和SEQ ID NO:106的BMPRII-Fc和ALK4-Fc融合多肽分别可共表达并从CHO细胞系中纯化,以产生包含BMPRII-Fc:ALK4-Fc的异聚体复合体。

[0889] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中论述了BMPRII-Fc融合多肽序列(SEQ ID NO:411和412)。为了促进BMPRII-Fc:ALK4-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入实施例16所示BMPRII-Fc多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:411和412的氨基酸序列。

[0890] 实施例1中论述了互补ALK4-Fc融合多肽的多肽序列(SEQ ID NO:403和404)。为了引导用SEQ ID NO:411和412的BMPRII-Fc融合多肽形成异二聚体,可将4个氨基酸取代引入实施例1所示ALK4融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:403和404的氨基酸序列。

[0891] SEQ ID NO:412和SEQ ID NO:404的BMPRII-Fc和ALK4-Fc融合多肽分别可共表达并从CHO细胞系中纯化,以产生包含BMPRII-Fc:ALK4-Fc的异聚体复合体。

[0892] 不同BMPRII-Fc:ALK4-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0893] 实施例23.与BMPRII-Fc同二聚体和ALK4-Fc同二聚体相比BMPRII-Fc:ALK4-Fc异二聚体的配体结合特征

[0894] 采用基于Biacore™的结合测定法以对上文所述BMPRII-Fc:ALK4-Fc异二聚体复合体的配体结合选择性与BMPRII-Fc和ALK4-Fc同二聚体复合体的进行比较。使用抗Fc抗体将BMPRII-Fc:ALK4-Fc异二聚体、BMPRII-Fc同二聚体和ALK4-Fc同二聚体独立俘获在系统中。注入配体,并使之在俘获的受体蛋白上流过。结果概括于下表,其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0895]

与 BMPRII-FC 同二聚体和 ALK4-FC 同二聚体相比 BMPRII-FC:ALK4-FC 异二聚体的配体结合特征									
配体	BMPRII-FC 同二聚体			ALK4-FC 同二聚体			BMPRII-FC:ALK4-FC 异二聚体		
	K_A (1/MS)	K_D (1/S)	K_D (PM)	K_A (1/MS)	K_D (1/S)	K_D (PM)	K_A (1/MS)	K_D (1/S)	K_D (PM)
激活素 A	瞬时*		>43000	$5.8 X10^5$	$1.2 X10^{-2}$	20000	$2.0 X10^6$	$2.2 X10^{-3}$	1100
激活素 B	$2.0 X10^7$	$7.5 X10^{-2}$	3800	无结合			$1.6 X10^6$	$2.6 X10^{-3}$	1700
激活素 AB	---	---	---	$4.4 X10^6$	$6.4 X10^{-3}$	1500	$3.6 X10^6$	$5.0 X10^{-4}$	140
BMP9	$1.2 X10^7$	$2.6 X10^{-2}$	2100	无结合			瞬时*		>140000
BMP10	$2.6 X10^7$	$2.5 X10^{-3}$	100	无结合			$8.0 X10^5$	$1.8 X10^{-3}$	2200
BMP15	$9.9 X10^6$	$2.8 X10^{-3}$	290	无结合			$2.8 X10^7$	$4.8 X10^{-2}$	1700
* 因相互作用的瞬时性质而不确定 --- 未测									

[0896] 这些比较结合数据表明BMPRII-Fc:ALK4-Fc异二聚体具有与BMPRII-Fc同二聚体或ALK4-Fc同二聚体不一同的配体结合选择性。BMPRII-Fc:ALK4-Fc异二聚体因以高或中等强度结合几种激活素配体而不同于两种同二聚体,且因仅弱结合BMP15而不同于BMPRII-Fc同二聚体。最值得注意的是,BMPRII-Fc:ALK4-Fc异二聚体与异二聚体配体激活素AB优先且牢固结合。参见图13。因此,BMPRII-Fc:ALK4-Fc异二聚体将意料不到地用于其中激活素A、激活素B、特别是激活素AB的拮抗作用是有利的且其中要避免BMP15(其大量参与排卵)的拮抗作用的某些治疗应用。

[0897] 实施例24.TGFβRII-Fc:ALK1-Fc异二聚体的产生

[0898] 申请人构建了包含人TGFβRII的短(典型)同种型和人ALK1的胞外域(其各自分别与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性TGFβRII-Fc:ALK1-Fc异聚体复合体。各个构建体分别被称为$TGF\beta RII_{SHORT}$-Fc融合多肽和ALK1-Fc融合多肽,本文提供各自的序列。

[0899] 通过类似于实施例1所述方法可引导异聚体$TGF\beta RII_{SHORT}$-Fc:ALK1-Fc的形成。在第一种方法中,改变一个Fc结构域以在相互作用面上引入阳离子氨基酸,同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。

[0900] $TGF\beta RII_{SHORT}$-Fc多肽序列(SEQ ID NO:127)见下:

```
   1   MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
  51   QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
 101   TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
 151   FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
 201   EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
 251   VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRKE
 301   MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LKSDGSFFLY
 351   SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
       (SEQ ID NO: 127)
```

[0901]

[0902] 前导序列和接头序列用下划线表示。为了促进TGFβRII$_{SHORT}$-Fc:ALK1-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示TGFβRII$_{SHORT}$-Fc融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:127的氨基酸序列。

[0903] 该TGFβRII$_{SHORT}$-Fc融合蛋白由下列核酸序列(SEQ ID NO:128)编码：

```
   1   ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51   AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101   TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
 151   CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
 201   ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
 251   AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
 301   ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
 351   TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
 401   CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
 451   TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAACTCA
 501   CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT
 551   TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
 601   GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA
 651   GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC
 701   CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC
 751   GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC
 801   CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG
 851   GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGAAGGAG
 901   ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC
 951   CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT
1001   ACAAGACCAC GCCTCCCGTG CTGAAGTCCG ACGGCTCCTT CTTCCTCTAT
1051   AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC
1101   ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC
1151   TCTCCCTGTC TCCGGGTAAA          (SEQ ID NO: 128)
```

[0904]

[0905]

[0906] 成熟的TGFβRII$_{SHORT}$-Fc融合多肽(SEQ ID NO:129)如下，并可任选提供从C端脱去

赖氨酸的序列。

[0907]

```
  1   TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51   ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101   MKEKKKPGET FFMCSCSSDE CNDNIIF参见YNTSNPDTGG GTHTCPPCPA
151   PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201   VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251   IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW
301   ESNGQPENNY KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351   LHNHYTQKSL SLSPGK      (SEQ ID NO: 129)
```

[0908] 实施例16中以SEQ ID NO:124-126提供互补ALK1-Fc融合多肽的多肽序列和其编码核酸序列。为了引导用SEQ ID NO:127和129的异二聚体形成TGFβRII$_{SHORT}$-Fc融合多肽,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入实施例16所示ALK1-Fc融合多肽的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:124和126的氨基酸序列。

[0909] SEQ ID NO:129和SEQ ID NO:126的TGFβRII$_{SHORT}$-Fc和ALK1-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含TGFβRII$_{SHORT}$-Fc:ALK1-Fc的异聚体复合体。

[0910] 可产生其中上文所述ALK1-Fc多肽(SEQ ID NO:126)与包含TGFβRII的长(A)同种型(TGFβRII$_{LONG}$)的胞外域替换短同种型的胞外域的Fc融合蛋白配对的变体TGFβRII-Fc:ALK1-Fc异聚体复合体。

[0911] TGFβRII$_{LONG}$-Fc多肽序列(SEQ ID NO:130)见下:

[0912]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51   RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
101   TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151   KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201   ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251   EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301   EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ VSLTCLVKGF YPSDIAVEWE
351   SNGQPENNYK TTPPVLKSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
401   HNHYTQKSLS LSPGK    (SEQ ID NO: 130)
```

[0913] 前导序列和接头序列用下划线表示。为了促进TGFβRII$_{LONG}$-Fc:ALK1-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示TGFβRII$_{LONG}$-Fc融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:130的氨基酸序列。

[0914] 该TGFβRII$_{LONG}$-Fc融合蛋白由下列核酸序列(SEQ ID NO:131)编码:

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101  ATGTGGAAAT GGAGGCCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
 151  AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
 201  CAACAACGGT GCAGTCAAGT TTCCACAACT GTGTAAATTT TGTGATGTGA
 251  GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
 301  ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
 351  GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCCAAGCTCC
```

[0915]

```
 401  CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
 451  AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
 501  TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
 551  ATCCTGACAC CGGTGGTGGA ACTCACACAT GCCCACCGTG CCCAGCACCT
 601  GAACTCCTGG GGGGACCGTC AGTCTTCCTC TTCCCCCCAA AACCCAAGGA
 651  CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG
 701  TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG
 751  GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC
 801  GTACCGTGTG GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG
 851  GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC
 901  GAGAAAACCA TCTCCAAAGC CAAAGGGCAG CCCCGAGAAC CACAGGTGTA
 951  CACCCTGCCC CCATCCCGGA AGGAGATGAC CAAGAACCAG GTCAGCCTGA
1001  CCTGCCTGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT GGAGTGGGAG
```

[0916]

```
1051  AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGAA
1101  GTCCGACGGC TCCTTCTTCC TCTATAGCAA GCTCACCGTG GACAAGAGCA
1151  GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG
1201  CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAA
      (SEQ ID NO: 131)
```

[0917] 成熟的TGFβRII$_{LONG}$-Fc融合多肽(SEQ ID NO:132)如下,并可任选提供从C端脱去赖氨酸的序列。

```
   1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
  51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
 101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
 151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
```

[0918]

```
 201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
 251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRK
 301  EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLKSDGSFFL
 351  YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
      (SEQ ID NO: 132)
```

[0919] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。

[0920] TGFβRII$_{SHORT}$-Fc多肽序列(SEQ ID NO:415)见下：

```
  1   MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51   QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101   TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151   FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201   EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251   VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPCREE
301   MTKNQVSLWC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
351   SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
      (SEQ ID NO: 415)
```

[0921]

[0922] 前导序列和接头序列用下划线表示。为了促进TGFβRII$_{SHORT}$-Fc:ALK1-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:415的氨基酸序列。

[0923] 成熟的TGFβRII$_{SHORT}$-Fc融合多肽(SEQ ID NO:416)如下，并可任选提供从C端脱去赖氨酸的序列。

[0924]

```
  1   TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51   ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101   MKEKKKPGET FFMCSCSSDE CNDNIIF参见YNTSNPDTGG GTHTCPPCPA
151   PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201   VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251   IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW
301   ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351   LHNHYTQKSL SLSPGK          (SEQ ID NO: 416)
```

[0925] 实施例16中论述了互补ALK1-Fc融合多肽的多肽序列(SEQ ID NO:413和414)。为了引导用SEQ ID NO:415和416的TGFβRII$_{SHORT}$-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入实施例16所示ALK1融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:413和414的氨基酸序列。

[0926] SEQ ID NO:416和SEQ ID NO:414的TGFβRII$_{SHORT}$-Fc和ALK1-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含的异聚体复合体TGFβRII-Fc:ALK1-Fc。

[0927] 可产生其中上文所述ALK1-Fc多肽(SEQ ID NO:414)与包含TGFβRII(TGFβRII$_{LONG}$)的长(A)同种型的胞外域替换短同种型的胞外域的Fc融合蛋白配对的变体TGFβRII-Fc:ALK1-Fc异聚体复合体。

[0928] TGFβRII$_{LONG}$-Fc多肽序列(SEQ ID NO:417)见下：

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
51 RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
101 TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151 KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
[0929] 201 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301 EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF YPSDIAVEWE
351 SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
401 HNHYTQKSLS LSPGK (SEQ ID NO: 417)

[0930] 前导序列和接头序列用下划线表示。为了促进TGFβRII$_{LONG}$-Fc:ALK1-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:417的氨基酸序列。

[0931] 成熟的TGFβRII$_{LONG}$-Fc融合多肽(SEQ ID NO:418)如下，并可任选提供从C端脱去赖氨酸的序列。

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
51 PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101 ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151 IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
[0932] 201 PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251 TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPCRE
301 EMTKNQVSLW CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
351 YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
(SEQ ID NO: 418)

[0933] SEQ ID NO:418和SEQ ID NO:414的TGFβRII$_{LONG}$-Fc和ALK1-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含TGFβRII$_{LONG}$-Fc:ALK1-Fc的异聚体复合体。

[0934] 不同TGFβRII-Fc:ALK1-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0935] 实施例25.与TGFβRII-Fc同二聚体和ALK1-Fc同二聚体相比TGFβRII-Fc:ALK1-Fc异二聚体的配体结合特征

[0936] 采用基于Biacore^{TM}的结合测定法以对上文所述TGFβRII$_{SHORT}$-Fc:ALK1-Fc异二聚体复合体的配体结合选择性与TGFβRII$_{SHORT}$-Fc和ALK1-Fc同二聚体复合体的进行比较。TGFβRII$_{SHORT}$-Fc:ALK1-Fc异二聚体、TGFβRII$_{SHORT}$-Fc同二聚体和ALK1-Fc同二聚体使用抗Fc抗体将独立俘获在系统中。注入配体，并使之在俘获的受体蛋白上流过。结果概括于下表，其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0937]

与 $TGFBRII_{SHORT}$-Fc 同二聚体和 ALK1-Fc 同二聚体相比 $TGFBRII_{SHORT}$-Fc:ALK1-Fc 异二聚体的配体结合特征									
配体	$TGFBRII_{SHORT}$-Fc 同二聚体			ALK1-Fc 同二聚体			$TGFBRII_{SHORT}$-Fc:ALK1-Fc 异二聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
BMP9	无结合			7.9×10^6	1.3×10^{-4}	16	2.1×10^7	2.2×10^{-3}	110
BMP10	无结合			1.7×10^7	1.1×10^{-4}	6	1.2×10^7	9.6×10^{-4}	78
TGFβ1	4.2×10^7	1.1×10^{-3}	25	无结合			瞬时*		>5300
TGFβ2	瞬时*		>44000	无结合			无结合		
TGFβ3	5.9×10^7	5.9×10^{-3}	99	无结合			瞬时*		>4700
* 因相互作用的瞬时性质而不确定									

[0938] 实施例26.TGFβRII_{SHORT}-Fc:ALK5-Fc异二聚体的产生

[0939] 申请人构建了包含人TGFβRII短(典型)同种型和人ALK5的胞外域(其各自分别与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性TGFβRII_{SHORT}-Fc:ALK5-Fc异聚体复合体。各个构建体分别被称为TGFβRII_{SHORT}-Fc融合多肽和ALK5-Fc融合多肽,本文提供各自的序列。

[0940] 通过类似于实施例1所述方法可引导异聚体TGFβRII_{SHORT}-Fc:ALK5-Fc的形成。在第一种方法中,改变一个Fc结构域以在相互作用面上引入阳离子氨基酸,同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。实施例24以SEQ ID NO:127-129提供TGFβRII_{SHORT}-Fc融合多肽的多肽序列和其编码核酸序列。为了促进TGFβRII_{SHORT}-Fc:ALK5-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入实施例24所示TGFβRII_{SHORT}-Fc融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:127和129的氨基酸序列。

[0941] 实施例11中以SEQ ID NO:139-141提供互补ALK5-Fc融合多肽的多肽序列和其编码核酸序列。为了引导用SEQ ID NO:127和129TGFβRII_{SHORT}-Fc融合多肽形成异二聚体,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入实施例11所示ALK5-Fc融合多肽的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:139和141的氨基酸序列。

[0942] SEQ ID NO:129和SEQ ID NO:141的TGFβRII_{SHORT}-Fc和ALK5-Fc融合多肽分别可共表达并从CHO细胞系中纯化,以产生包含TGFβRII_{SHORT}-Fc:ALK5-Fc的异聚体复合体。

[0943] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例24中论述了TGFβRII_{SHORT}-Fc融合多肽序列(SEQ ID NO:415-416)。为了促进TGFβRII_{SHORT}-Fc:ALK5-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入实施例24所示TGFβRII_{SHORT}-Fc多肽的Fc结构域中。可任选提供从C

端脱去赖氨酸的SEQ ID NO:415-416的氨基酸序列。

[0944] 实施例11中论述了互补ALK5-Fc融合多肽的多肽序列(SEQ ID NO:423-424)。为了引导用SEQ ID NO:415-416的TGFβRII$_{SHORT}$-Fc融合多肽形成异二聚体,可将4个氨基酸取代引入实施例11所示ALK5融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:423-424的氨基酸序列。

[0945] SEQ ID NO:416和SEQ ID NO:424的TGFβRII$_{SHORT}$-Fc和ALK5-Fc融合多肽分别可共表达并从CHO细胞系中纯化,以产生包含TGFβRII$_{SHORT}$-Fc:ALK5-Fc的异聚体复合体。

[0946] 不同TGFβRII$_{SHORT}$-Fc:ALK5-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0947] 实施例27.TGFβRII$_{LONG}$-Fc:ALK5-Fc异二聚体的产生

[0948] 申请人构建了包含人TGFβRII的长(A)同种型的胞外域和人ALK5的胞外域(其各自分别与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性TGFβRII$_{LONG}$-Fc:ALK5-Fc异聚体复合体。各个构建体分别被称为TGFβRII$_{LONG}$-Fc融合多肽和ALK5-Fc融合多肽,本文提供各自的序列。

[0949] 通过类似于实施例1所述方法可引导异聚体TGFβRII$_{LONG}$-Fc:ALK5-Fc的形成。在第一种方法中,改变一个Fc结构域以在相互作用面上引入阳离子氨基酸,同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。上文实施例24中以SEQ ID NO:130-132提供TGFβRII$_{LONG}$-Fc融合多肽的多肽序列和其编码核酸序列。为了促进TGFβRII$_{LONG}$-Fc:ALK5-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入实施例24所示TGFβRII$_{LONG}$-Fc融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:130和132的氨基酸序列。

[0950] 实施例11中以SEQ ID NO:139-141提供互补ALK5-Fc融合多肽的多肽序列和其编码核酸序列。为了引导用SEQ ID NO:130和132的TGFβRII$_{LONG}$-Fc融合多肽形成异二聚体,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入实施例11所示ALK5-Fc融合多肽的Fc结构域中。可任选提供C端加入赖氨酸的SEQ ID NO:139和142的氨基酸序列。

[0951] SEQ ID NO:132和SEQ ID NO:141的TGFβRII$_{LONG}$-Fc和ALK5-Fc融合多肽分别可共表达并从CHO细胞系中纯化,以产生包含TGFβRII$_{LONG}$-Fc:ALK5-Fc的异聚体复合体。

[0952] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例24中论述了TGFβRII$_{LONG}$-Fc融合多肽序列(SEQ ID NO:417-418)。为了促进TGFβRII$_{LONG}$-Fc:ALK5-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入实施例24所示TGFβRII$_{LONG}$-Fc多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:417-418的氨基酸序列。

[0953] 实施例11中论述了互补ALK5-Fc融合多肽的多肽序列(SEQ ID NO:423-424)。为了引导用SEQ ID NO:417-418的TGFβRII$_{LONG}$-Fc融合多肽形成异二聚体,可将4个氨基酸取代引入实施例11所示ALK5融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:423-424的氨基酸序列。

[0954] SEQ ID NO:418和SEQ ID NO:424的TGFβRII$_{LONG}$-Fc和ALK5-Fc融合多肽分别可共

表达并从CHO细胞系中纯化，以产生包含$TGF\beta RII_{LONG}$-Fc:ALK5-Fc的异聚体复合体。

[0955] 不同$TGF\beta RII_{LONG}$-Fc:ALK5-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0956] 实施例28.与TGFβRII-Fc同二聚体和ALK5-Fc同二聚体相比TGFβRII-Fc:ALK5-Fc异二聚体的活性特征

[0957] 采用基于Biacore™的结合测定法以对描述于实施例26-27的$TGF\beta RII_{SHORT}$-Fc:ALK5-Fc和$TGF\beta RII_{LONG}$-Fc:ALK5-Fc异二聚体复合体的配体结合选择性与$TGF\beta RII_{SHORT}$-Fc和ALK5-Fc同二聚体复合体的进行比较。使用抗Fc抗体将异聚体或同聚体蛋白质复合体独立俘获在系统中。注入配体，并使之在俘获的受体蛋白上流过。结果概括于下表，其中最能表明有效配体阱的配体解离速率(k_d)用灰色阴影标示。

[0958]

与 TGFβRII-Fc 同二聚体和 ALK5-Fc 同二聚体相比 TGFβRII-Fc:ALK5-Fc 异二聚体的配体结合特征												
配体	ALK5-Fc 同二聚体			$TGF\beta RII_{SHORT}$-Fc 同二聚体			$TGF\beta RII_{SHORT}$-Fc:ALK5-Fc 异二聚体*			$TGF\beta RII_{LONG}$-Fc:ALK5-Fc 异二聚体*		
	k_a	k_d	K_D	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
TGFβ1	无结合			5.6×10^7	1.1×10^{-3}	20	1.4×10^8	1.7×10^{-3}	12	6.6×10^7	9.2×10^{-4}	14
TGFβ2	无结合			2.1×10^5	2.2×10^{-3}	11000	6.6×10^6	2.9×10^{-6}	0.4	4.2×10^6	2.8×10^{-7}	0.07
TGFβ3	无结合			1.9×10^7	1.4×10^{-3}	71	2.7×10^7	1.0×10^{-3}	38	2.7×10^7	1.0×10^{-3}	38
* 表明大量的蛋白质部分是无活性的低信号												

[0959]

[0960] 这些比较结合数据表明TGFβRII-Fc:ALK5-Fc异二聚体的配体结合特征显著不同于TGFβRII-Fc同二聚体，且不同于ALK5-Fc同二聚体的配体结合特征，后者不结合任何配体。根据平衡解离常数(K_D)，TGFβRII-Fc同二聚体以比TGFβ1高得多的亲和力结合TGFβ1和TGFβ3，虽然3种TGFβ配体的解离速率相似。相比之下，TGFβRII-Fc:ALK5-Fc异二聚体显示对TGFβ2超过TGFβ1/TGFβ3的高选择性。具体地讲，$TGF\beta RII_{LONG}$-Fc:ALK5-Fc异二聚体以比TGFβRII-Fc同二聚体高约5个数量级的亲和力和比之慢约4个数量级的解离速率结合TGFβ2。$TGF\beta RII_{LONG}$-Fc:ALK5-Fc异二聚体比含有短同种型的异二聚体更牢固地结合TGFβ2。参见图14。TGFβRII-Fc:ALK5-Fc异二聚体无一能够结合BMP9或BMP10(数据未显示)，这将这些TGFβRII-Fc:ALK5-Fc异二聚体与TGFβRII-Fc:ALK1-Fc异二聚体区分开来(参见实施例25)。两种TGFβRII-Fc:ALK5-Fc异二聚体的传感图显示低信号幅度，表明了各个蛋白质的大部分是无活性的。

[0961] 为了更好的解释通过表面等离子体共振获得的这些数据，在A549细胞采用报道基

因测定法测定TGFβRII融合蛋白抑制TGFβ1、TGFβ2和TGFβ3的活性的能力。该测定法基于用报道分子质粒pGL3(CAGA)12-萤火虫萤光素酶(Dennler等,1998,EMBO17:3091-3100)和pRLCMV-海肾萤光素酶转染的人肺癌细胞系,后者用于控制转染效率。CAGA基序存在于TGFβ-响应基因的启动子(例如PAI-1)中,因此该载体对于通过SMAD2和SMAD3发出信号的因子具有通用性。

[0962] 在测定的第一天,将A549细胞(ATCC®:CCL-185™)以6.5x10^{4}个细胞/孔分布在48孔板中,并孵育过夜。所有的孵育都在组织培养箱中在37℃和5%CO_2下,除非另有说明。在第二天,将含有10μg pGL3(CAGA)12-萤火虫萤光素酶、100ng pRLCMV-海肾萤光素酶、30μL X-tremeGENE9(Roche Applied Science)和970μL OptiMEM(Invitrogen)的溶液在室温下预先孵育30分钟,然后加入24mL补充0.1%BSA的Eagle极限必需培养基(EMEM,ATCC®)中。从铺板的细胞中取出培养基,将该转染混合物施加到细胞(500μl/孔)中用于过夜孵育。在第三天,除去培养基,使细胞与按下文所述制备的配体和抑制剂的混合物一起孵育过夜。

[0963] 在48孔板中在200μL体积的测定缓冲液(EMEM+0.1%BSA)中制备试验品的系列稀释液。加入等体积的含有试验配体的测定缓冲液,获得等于之前确定的EC_{50}的最终配体浓度。人TGFβ1、TGFβ2和TGFβ3获自PeproTech。将试验溶液孵育30分钟,然后将250μL的混合物加入转染的细胞中。一式两份测定试验品的各个浓度。在与试验溶液一起孵育过夜后,将细胞用磷酸缓冲盐水冲洗,然后用被动裂解缓冲液(Promega E1941)裂解,并在-70℃下保存过夜。在第4天和最后一天,在轻轻振荡时使板升温至室温。将细胞裂解物转移到化学发光板(96孔)中,在发光计中用双重萤光素酶报道分子测定系统(Promega E1980)的试剂分析,测定归一化萤光素酶活性。

[0964] 该测定法用来比较TGFβRII融合蛋白变体通过TGFβRII配体抑制细胞信号转导的能力。结果见下表。

[0965]

A549 细胞中 TGFβRII 融合蛋白的抑制活性

构建体	IC_{50} (pM)		
	TGFβ1 (640 pg/mL)	TGFβ2 (480 pg/mL)	TGFβ3 (270 pg/mL)
$TGF\beta RII_{SHORT}$-Fc 同二聚体	90	---	9
$TGF\beta RII_{SHORT}$-Fc:ALK5-Fc 异二聚体*	< 350*	~ 200*	< 90*
$TGF\beta RII_{LONG}$-Fc:ALK5-Fc 异二聚体	204	154	35

--- 无抑制(在高达 10 nM 的浓度下测试)

* 因所测浓度范围所致值不精确

[0966] 用TGFβRII-Fc同二聚体的结果与之前有关野生型$TGF\beta RII_{SHORT}$-Fc和$TGF\beta RII_{LONG}$-Fc同二聚体的报道(del Re等,J Biol Chem279:22765,2004)一致。在该实验中,$TGF\beta RII_{SHORT}$-Fc同二聚体有效地抑制TGFβ1和TGFβ3,但是在高达10nM的同二聚体浓度下不能抑

制TGFβ2。这个结果与TGFβ2结合TGFβRII-Fc同二聚体的低亲和力一致，但奇怪地与其慢的解离速率不一致(参见上文的结合结果)。相比之下，TGFβRII-Fc:ALK5-Fc异二聚体在细胞环境中有效地抑制所有3种TGFβ配体。因此，TGFβRII-Fc:ALK5-Fc异二聚体将意料不到地用于其中TGFβ2优先的拮抗作用—或TGFβ1、TGFβ2和TGFβ3的综合拮抗作用—是有利的某些治疗应用。

[0967] 实施例29.MISRII-Fc:ALK3-Fc异二聚体的产生

[0968] 可产生包含其各自可与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合的人MISRII和人ALK3的胞外域的可溶性MISRII-Fc:ALK3-Fc异聚体复合体。各个构建体分别被称为MISRII-Fc融合多肽和ALK3-Fc融合多肽。

[0969] 通过类似于实施例1所述方法可引导异聚体MISRII-Fc:ALK3-Fc的形成。在第一种方法中，改变一个Fc结构域以在相互作用面上引入阳离子氨基酸，同时改变另一个Fc结构域以在相互作用面上引入阴离子氨基酸。

[0970] MISRII-Fc多肽序列(SEQ ID NO:133)见下：

[0971]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
 51  DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101  PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151  SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
201  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ
301  VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLKSDG SFFLYSKLTV
351  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 133)
```

[0972] 前导序列和接头序列用下划线表示。为了促进MISRII-Fc:ALK3-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示MISRII融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:133的氨基酸序列。

[0973] 成熟的MISRII-Fc融合多肽(SEQ ID NO:135)如下，并可任选提供从C端脱去赖氨酸的序列。

[0974]
```
  1  PPNRRTCVFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51  TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
101  ANYSHLPPPG SPGTPGSQGP QAAPGESIWM ALTGGGTHTC PPCPAPELLG
151  GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
201  AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
251  SKAKGQPREP QVYTLPPSRK EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ
301  PENNYKTTPP VLKSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
351  TQKSLSLSPG K    (SEQ ID NO: 135)
```

[0975] 在此第一种方法中，上文实施例4中以SEQ ID NO:115-117提供互补ALK3-Fc融合蛋白的多肽序列和其编码核酸序列。

[0976] SEQ ID NO:135和SEQ ID NO:117的MISRII-Fc和ALK3-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含MISRII-Fc:ALK3-Fc的异聚体复合体。可通过使上文所述

ALK3-Fc蛋白(SEQ ID NO:117)与包含MISRII的胞外域同种型2或MISRII同种型3的胞外域替换MISRII同种型1的胞外域的Fc融合蛋白配对,产生类似的异聚体复合体。

[0977] 在使用不对称Fc融合蛋白促进异多聚体复合体的形成的第二种方法中,可改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。

[0978] MISRII-Fc多肽序列(SEQ ID NO:419)见下:

[0979]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
 51  DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101  PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151  SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
201  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ
301  VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
351  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
```

[0980] (SEQ ID NO: 419)

[0981] 前导序列和接头序列用下划线表示。为了促进MISRII-Fc:ALK3-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:419的氨基酸序列。

[0982] 成熟的MISRII-Fc融合多肽(SEQ ID NO:420)如下,并可任选提供从C端脱去赖氨酸的序列。

[0983]
```
  1  PPNRRTCVFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51  TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
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 VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
351  TQKSLSLSPG K(SEQ ID NO: 420)
```

[0984] 在此第二种方法中,实施例4中以SEQ ID NO:407-408提供互补ALK3-Fc融合蛋白的多肽序列和其编码核酸序列。

[0985] SEQ ID NO:420和SEQ ID NO:408的MISRII-Fc和ALK3-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含MISRII-Fc:ALK3-Fc的异聚体复合体。可通过使上文所述ALK3-Fc蛋白(SEQ ID NO:408)与Fc融合蛋白包含MISRII同种型2的胞外域或MISRII同种型3的胞外域替换上文所述MISRII同种型1的胞外域配对,产生类似的异聚体复合体。

[0986] 不同MISRII-Fc:ALK3-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[0987] 实施例30.ActRIIA-Fc:ActRIIB-Fc异二聚体的产生

[0988] 可产生包含人ActRIIA和人ActRIIB的胞外域(其各自与具有位于胞外域和Fc结构

域之间的接头的Fc结构域融合)的可溶性ActRIIA-Fc:ActRIIB-Fc异聚体复合体。各个构建体分别被称为ActRIIA-Fc和ActRIIB-Fc融合蛋白。

[0989] 通过类似于实施例1所述方法可引导异聚体ActRIIA-Fc:ActRIIB-Fc的形成。

[0990] 静电方法

[0991] 在第一种方法中,上文实施例14中以SEQ ID NO:118-120提供ActRIIA-Fc融合蛋白的多肽序列和其编码核酸序列。

[0992] 互补ActRIIB-Fc融合蛋白的多肽序列(SEQ ID NO:153)使用TPA前导序列,且列举如下:

[0993]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNG关键KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
301  EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK          (SEQ ID NO: 153)
```

[0994] 前导序列和接头序列用下划线表示。为了促进ActRIIA-Fc:ActRIIB-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:153的氨基酸序列。

[0995] 成熟的ActRIIB-Fc融合蛋白序列(SEQ ID NO:154)如下,并可任选提供从C端脱去赖氨酸的序列。

[0996]
```
  1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
251  REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYDTT PPVLDSDGSF
301  FLYSDLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
```

[0997] (SEQ ID NO: 154)

[0998] SEQ ID NO:120和SEQ ID NO:154的ActRIIA-Fc和ActRIIB-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:ActRIIB-Fc的异聚体复合体。

[0999] 反向静电方法

[1000] 在反向方法中,与上文描述的第一种方法相比,受体胞外域同时与Fc相互作用对的对立成员连接。在该实施例中,ActRIIA-Fc融合蛋白的备选多肽序列(SEQ ID NO:151)使用TPA前导序列,且列举如下。

[1001]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51   GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101   DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151   CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201   VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251   PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
301   VEWESNGQPE NNYDTTPPVL DSDGSFFLYS DLTVDKSRWQ QGNVFSCSVM
351   HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 151)
```

[1002] 前导序列和接头序列用下划线表示。为了促进ActRIIA-Fc:ActRIIB-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:151的氨基酸序列。

[1003] 成熟的ActRIIA-Fc融合蛋白序列(SEQ ID NO:152)如下，并可任选提供从C端脱去赖氨酸的序列。

[1004]

```
  1   ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51   IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101   EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151   SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201   SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
251   SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYDT TPPVLDSDGS
301   FFLYSDLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
      (SEQ ID NO: 152)
```

[1005] 实施例1中以SEQ ID NO:100-102提供ActRIIB-Fc融合蛋白的多肽序列和其编码核酸序列。

[1006] SEQ ID NO:152和SEQ ID NO:102的ActRIIA-Fc和ActRIIB-Fc融合蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:ActRIIB-Fc的变体异聚体复合体。

[1007] 疏水方法

[1008] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例14中以SEQ ID NO:409-410提供ActRIIA-Fc融合蛋白的多肽序列。

[1009] ActRIIB-Fc融合多肽的互补形式(SEQ ID NO:453)如下：

[1010]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51   GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101   ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151   PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNG关键KCKVSNKALP
251   APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV KGFYPSDIAV
301   EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH
351   EALHNHYTQK SLSLSPGK          (SEQ ID NO: 453)
```

[1011] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:409-410的ActRIIA-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入上文双下划线所示ActRIIB-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:453的氨基酸序列。

[1012] 成熟的ActRIIB-Fc融合蛋白序列(SEQ ID NO:454)如下，并可任选提供从C端脱去赖氨酸的序列。

[1013]
```
  1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVCTLPPS
251  REEMTKNQVS LSCAVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301  FLVSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
     (SEQ ID NO: 454)
```

[1014] SEQ ID NO:410和SEQ ID NO:454的ActRIIA-Fc和ActRIIB-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:ActRIIB-Fc的变体异聚体复合体ActRIIA-Fc:ActRIIB-Fc。

[1015] 反向疏水方法

[1016] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，按紧接的上文所述，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而，与上述方法相比，该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，ActRIIA-Fc融合蛋白的备选多肽序列(SEQ ID NO:451)使用TPA前导序列，且列举如下。

[1017]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGAAILGRS ETQECLFFNA NWEKDRTNQT
 51  GVEPCYGDKD KRRHCFATWK NISGSIEIVK QGCWLDDINC YDRTDCVEKK
101  DSPEVYFCCC EGNMCNEKFS YFPEMEVTQP TSNPVTPKPP TGGGTHTCPP
151  CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
201  VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
251  PAPIEKTISK AKGQPREPQV CTLPPSREEM TKNQVSLSCA VKGFYPSDIA
301  VEWESNGQPE NNYKTTPPVL DSDGSFFLVS KLTVDKSRWQ QGNVFSCSVM
351  HEALHNHYTQ KSLSLSPGK          (SEQ ID NO: 451)
```

[1018] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO401-402的ActRIIB-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入上文双下划线所示ActRIIA-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:451的氨基酸序列。

[1019] 成熟的ActRIIA-Fc融合蛋白序列(SEQ ID NO:452)如下，并可任选提供从C端脱去赖氨酸的序列。

[1020]

```
  1   ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
 51   IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
101   EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI
151   SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
201   SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVCTLPP
251   SREEMTKNQV SLSCAVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
301   FFLVSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
      (SEQ ID NO: 452)
```

[1021] 实施例1中以SEQ ID NO:401-402提供ActRIIB-Fc融合蛋白的多肽序列。

[1022] SEQ ID NO:452和SEQ ID NO:402的ActRIIA-Fc和ActRIIB-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:ActRIIB-Fc的变体异聚体复合体。

[1023] 不同ActRIIA-Fc:ActRIIB-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1024] 实施例31.ActRIIA-Fc:BMPRII-Fc异二聚体的产生

[1025] 可产生包含人ActRIIA和人BMPRII的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIA-Fc:BMPRII-Fc异聚体复合体。各个构建体被称为ActRIIA-Fc和BMPRII-Fc融合蛋白,分别。

[1026] 通过与描述于实施例30类似的方法可引导异聚体ActRIIA-Fc:BMPRII-Fc的形成。

[1027] 静电方法

[1028] 在第一种方法中,实施例14中以SEQ ID NO:118-120提供ActRIIA-Fc融合蛋白的多肽序列和其编码核酸序列。

[1029] 互补BMPRII-Fc融合蛋白的多肽序列(SEQ ID NO:155)使用TPA前导序列,且列举如下:

[1030]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51   HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101   TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151   GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201   EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251   KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG
301   FYPSDIAVEW ESNGQPENNY DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN
351   VFSCSVMHEA LHNHYTQKSL SLSPGK        (SEQ ID NO: 155)
```

[1031] 前导序列和接头序列用下划线表示。为了促进ActRIIA-Fc:BMPRII-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:155的氨基酸序列。

[1032] 成熟的BMPRII-Fc融合蛋白序列(SEQ ID NO:156)如下,并可任选提供从C端脱去赖氨酸的序列。

[1033]

```
  1   SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
 51   INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
101   FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
151   KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201   YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251   PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP
301   PVLDSDGSFF LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351   GK        (SEQ ID NO: 156)
```

[1034] SEQ ID NO:120和SEQ ID NO:156的ActRIIA-Fc和BMPRII-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:BMPRII-Fc的异聚体复合体。

[1035] 反向静电方法

[1036] 在反向方法中,与上文描述的第一种方法相比,受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例30中以SEQ ID NO:151-152提供ActRIIA-Fc融合蛋白的备选多肽序列。

[1037] 实施例16中以SEQ ID NO:121-123提供BMPRII-Fc融合蛋白的多肽序列和其编码核酸序列。

[1038] SEQ ID NO:152和SEQ ID NO:123的ActRIIA-Fc和BMPRII-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:BMPRII-Fc的变体异聚体复合体。

[1039] 疏水方法

[1040] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例14中以SEQ ID NO409-410提供ActRIIA-Fc融合蛋白的多肽序列。

[1041] BMPRII-Fc融合多肽的互补形式(SEQ ID NO:455)如下:

[1042]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGASQNQER LCAFKDPYQQ DLGIGESRIS
 51   HENGTILCSK GSTCYGLWEK SKGDINLVKQ GCWSHIGDPQ ECHYEECVVT
101   TTPPSIQNGT YRFCCCSTDL CNVNFTENFP PPDTTPLSPP HSFNRDETGG
151   GTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
201   EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
251   KVSNKALPAP IEKTISKAKG QPREPQVCTL PPSREEMTKN QVSLSCAVKG
301   FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN
351   VFSCSVMHEA LHNHYTQKSL SLSPGK      (SEQ ID NO: 455)
```

[1043] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:409-410的ActRIIA-Fc融合多肽形成异二聚体,可将4个氨基酸取代引入上文双下划线所示BMPRII-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:455的氨基酸序列。

[1044] 成熟的BMPRII-Fc融合蛋白序列(SEQ ID NO:456)如下,并可任选提供从C端脱去赖氨酸的序列。

[1045]

```
  1   SQNQERLCAF KDPYQQDLGI GESRISHENG TILCSKGSTC YGLWEKSKGD
 51   INLVKQGCWS HIGDPQECHY EECVVTTTPP SIQNGTYRFC CCSTDLCNVN
101   FTENFPPPDT TPLSPPHSFN RDETGGGTHT CPPCPAPELL GGPSVFLFPP
151   KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201   YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251   PQVCTLPPSR EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP
301   PVLDSDGSFF LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351   GK       (SEQ ID NO: 456)
```

[1046] SEQ ID NO:410和SEQ ID NO:456的ActRIIA-Fc和BMPRII-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:BMPRII-Fc的变体异聚体复合体。

[1047] 反向疏水方法

[1048] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,按紧接的上文所述,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而,与上述方法相比,该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例30中以SEQ ID NO:451-452提供ActRIIA-Fc融合蛋白的备选多肽序列。

[1049] 实施例16中以SEQ ID NO:411-412提供BMPRII-Fc融合蛋白的多肽序列。

[1050] SEQ ID NO:452和SEQ ID NO:412的ActRIIA-Fc和BMPRII-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:BMPRII-Fc的变体异聚体复合体。

[1051] 不同ActRIIA-Fc:BMPRII-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1052] 实施例32.ActRIIA-Fc:MISRII-Fc异二聚体的产生

[1053] 可产生包含人ActRIIA和人MISRII的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIA-Fc:MISRII-Fc异聚体复合体。各个构建体分别被称为ActRIIA-Fc和MISRII-Fc融合蛋白。

[1054] 可通过类似于实施例30所述方法引导异聚体ActRIIA-Fc:MISRII-Fc的形成。

[1055] 静电方法

[1056] 在第一种方法中,实施例14中以SEQ ID NO:118-120提供ActRIIA-Fc融合蛋白的多肽序列和其编码核酸序列。

[1057] 互补MISRII-Fc融合蛋白的多肽序列(SEQ ID NO:161)使用TPA前导序列,且列举如下:

[1058]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
 51   DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101   PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151   SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
201   VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251   WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ
301   VSLTCLVKGF YPSDIAVEWE SNGQPENNYD TTPPVLDSDG SFFLYSDLTV
351   DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 161)
```

[1059] 前导序列和接头序列用下划线表示。为了促进ActRIIA-Fc:MISRII-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:161的氨基酸序列。

[1060] 成熟的MISRII-Fc融合蛋白序列(SEQ ID NO:162)如下，并可任选提供从C端脱去赖氨酸的序列。

[1061]
```
  1   PPNRRTCVFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51   TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
101   ANYSHLPPPG SPGTPGSQGP QAAPGESIWM ALTGGGTHTC PPCPAPELLG
151   GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
201   AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
251   SKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ
301   PENNYDTTPP VLDSDGSFFL YSDLTVDKSR WQQGNVFSCS VMHEALHNHY
351   TQKSLSLSPG K        (SEQ ID NO: 162)
```

[1062] SEQ ID NO:120和SEQ ID NO:162的ActRIIA-Fc和MISRII-Fc融合蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:MISRII-Fc的异聚体复合体。

[1063] 反向静电方法

[1064] 在反向方法中，与上文描述的第一种方法相比，受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例30中以SEQ ID NO:151和152提供ActRIIA-Fc融合蛋白的备选多肽序列。

[1065] 实施例29中以SEQ ID NO:133和135提供MISRII-Fc融合蛋白的多肽序列。

[1066] SEQ ID NO:152和SEQ ID NO:135的ActRIIA-Fc和MISRII-Fc融合蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:MISRII-Fc的变体异聚体复合体。

[1067] 疏水方法

[1068] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例14中以SEQ ID NO409-410提供ActRIIA-Fc融合蛋白的多肽序列。

[1069] MISRII-Fc融合多肽的互补形式(SEQ ID NO:457)如下：

[1070]
```
  1   MDAMKRGLCC VLLLCGAVFV SPGAPPNRRT CVFFEAPGVR GSTKTLGELL
 51   DTGTELPRAI RCLYSRCCFG IWNLTQDRAQ VEMQGCRDSD EPGCESLHCD
101   PSPRAHPSPG STLFTCSCGT DFCNANYSHL PPPGSPGTPG SQGPQAAPGE
151   SIWMALTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
201   VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
251   WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSREEMTKNQ
301   VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSKLTV
351   DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
      (SEQ ID NO: 457)
```

[1071] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:409-410的ActRIIA-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入上文双下划线所示MISRII-Fc融合多

肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:457的氨基酸序列。

[1072] 成熟的MISRII-Fc融合蛋白序列(SEQ ID NO:458)如下,并可任选提供从C端脱去赖氨酸的序列。

[1073]

```
  1  PPNRRTCVFF EAPGVRGSTK TLGELLDTGT ELPRAIRCLY SRCCFGIWNL
 51  TQDRAQVEMQ GCRDSDEPGC ESLHCDPSPR AHPSPGSTLF TCSCGTDFCN
101  ANYSHLPPPG SPGTPGSQGP QAAPGESIWM ALTGGGTHTC PPCPAPELLG
151  GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
201  AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
251  SKAKGQPREP QVCTLPPSRE EMTKNQVSLS CAVKGFYPSD IAVEWESNGQ
301  PENNYKTTPP VLDSDGSFFL VSKLTVDKSR WQQGNVFSCS VMHEALHNHY
351  TQKSLSLSPG K(SEQ ID NO: 458)
```

[1074] SEQ ID NO:410和SEQ ID NO:458的ActRIIA-Fc和MISRII-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:MISRII-Fc的变体异聚体复合体。

[1075] 反向疏水方法

[1076] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,按紧接的上文所述,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而,与上述方法相比,该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例30中以SEQ ID NO:451-452提供ActRIIA-Fc融合蛋白的备选多肽序列。

[1077] 实施例29中以SEQ ID NO:419-420提供MISRII-Fc融合蛋白的多肽序列。

[1078] SEQ ID NO:452和SEQ ID NO:420的ActRIIA-Fc和MISRII-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:MISRII-Fc的变体异聚体复合体。

[1079] 不同ActRIIA-Fc:MISRII-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1080] 实施例33.ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc异二聚体的产生

[1081] 可产生包含人ActRIIA和人TGFβRII$_{SHORT}$的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc异聚体复合体。各个构建体分别被称为ActRIIA-Fc和TGFβRII$_{SHORT}$-Fc融合蛋白。

[1082] 可通过类似于实施例30所述方法引导异聚体ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc的形成。

[1083] 静电方法

[1084] 在第一种方法中,实施例14中以SEQ ID NO:118-120提供ActRIIA-Fc融合蛋白的多肽序列和其编码核酸序列。

[1085] 互补TGFβRII$_{SHORT}$-Fc融合蛋白的多肽序列(SEQ ID NO:157)使用TPA前导序列,且列举如下:

[1086]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
 51   QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
101   TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
151   FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
201   EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
251   VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE
301   MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYDTTPPV LDSDGSFFLY
351   SDLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
```

[1087] (SEQ ID NO: 157)

[1088] 前导序列和接头序列用下划线表示。为了促进ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:157的氨基酸序列。

[1089] 成熟的TGFβRII$_{SHORT}$-Fc融合蛋白序列(SEQ ID NO:158)如下，并可任选提供从C端脱去赖氨酸的序列。

[1090]

```
  1   TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
 51   ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
101   MKEKKKPGET FFMCSCSSDE CNDNIIF参见YNTSNPDTGG GTHTCPPCPA
151   PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201   VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251   IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW
301   ESNGQPENNY DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA
351   LHNHYTQKSL SLSPGK(SEQ ID NO: 158)
```

[1091] SEQ ID NO:120和SEQ ID NO:158的ActRIIA-Fc和TGFβRII$_{SHORT}$-Fc融合蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc的异聚体复合体。

[1092] 反向静电方法

[1093] 在反向方法中，与上文描述的第一种方法相比，受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例30中以SEQ ID NO:151-152提供ActRIIA-Fc融合蛋白的备选多肽序列。

[1094] 实施例24中以SEQ ID NO:127-129提供TGFβRII$_{SHORT}$-Fc融合蛋白的多肽序列和其编码核酸序列。

[1095] SEQ ID NO:152和SEQ ID NO:129的ActRIIA-Fc和TGFβRII$_{SHORT}$-Fc融合蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc的变体异聚体复合体。

[1096] 疏水方法

[1097] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例14中以SEQ ID NO409-410提供ActRIIA-Fc融合蛋白的多肽序列。

[1098] TGFβRII$_{SHORT}$-Fc融合多肽的互补形式(SEQ ID NO:459)如下：

```
   1   MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP
  51   QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE
 101   TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII
 151   FSEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
 201   EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT
 251   VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VCTLPPSREE
 301   MTKNQVSLSC AVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLV
 351   SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
       (SEQ ID NO: 459)
```

[1099]

[1100] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:409-410的ActRIIA-Fc融合多肽形成异二聚体,可将4个氨基酸取代引入上文双下划线所示TGFβRII$_{SHORT}$-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:459的氨基酸序列。

[1101] 成熟的TGFβRII$_{SHORT}$-Fc融合蛋白序列(SEQ ID NO:460)如下,并可任选提供从C端脱去赖氨酸的序列。

```
   1   TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
  51   ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
 101   MKEKKKPGET FFMCSCSSDE CNDNIIF参见YNTSNPDTGG GTHTCPPCPA
 151   PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
 201   VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
 251   IEKTISKAKG QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW
 301   ESNGQPENNY KTTPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA
 351   LHNHYTQKSL SLSPGK(SEQ ID NO: 460)
```

[1102]

[1103] SEQ ID NO:410和SEQ ID NO:460的ActRIIA-Fc和TGFβRII$_{SHORT}$-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc的变体异聚体复合体。

[1104] 反向疏水方法

[1105] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,按紧接的上文所述,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而,与上述方法相比,该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例30中以SEQ ID NO:451-452提供ActRIIA-Fc融合蛋白的备选多肽序列。

[1106] 实施例24中以SEQ ID NO:415-416提供TGFβRII$_{SHORT}$-Fc融合蛋白的多肽序列。

[1107] SEQ ID NO:452和SEQ ID NO:416的ActRIIA-Fc和TGFβRII$_{SHORT}$-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc的变体异聚体复合体。

[1108] 不同ActRIIA-Fc:TGFβRII$_{SHORT}$-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1109] 实施例34.ActRIIA-Fc:TGFβRII$_{LONG}$-Fc异二聚体的产生

[1110] 可产生包含人ActRIIA和人TGFβRII$_{LONG}$的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ActRIIA-Fc:TGFβRII$_{LONG}$-Fc异聚体复合体。各个构建体分别被称为ActRIIA-Fc和TGFβRII$_{LONG}$-Fc融合蛋白。

[1111] 可通过类似于实施例30所述方法引导异聚体ActRIIA-Fc:TGFβRII$_{LONG}$-Fc的形成。

[1112] 静电方法

[1113] 在第一种方法中,实施例14中以SEQ ID NO:118-120提供ActRIIA-Fc融合蛋白的多肽序列和其编码核酸序列。

[1114] 互补TGFβRII$_{LONG}$-Fc融合蛋白的多肽序列(SEQ ID NO:159)使用TPA前导序列,且列举如下:

[1115]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51  RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
101  TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151  KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201  ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251  EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301  EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE
351  SNGQPENNYD TTPPVLDSDG SFFLYSDLTV DKSRWQQGNV FSCSVMHEAL
401  HNHYTQKSLS LSPGK      (SEQ ID NO: 159)
```

[1116]

[1117] 前导序列和接头序列用下划线表示。为了促进ActRIIA-Fc:TGFβRII$_{LONG}$-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将赖氨酸用天冬氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:159的氨基酸序列。

[1118] 成熟的TGFβRII$_{LONG}$-Fc融合蛋白序列(SEQ ID NO:160)如下,并可任选提供从C端脱去赖氨酸的序列。

[1119]
```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE
301  EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYDTTPP VLDSDGSFFL
351  YSDLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
     (SEQ ID NO: 160)
```

[1120] SEQ ID NO:120和SEQ ID NO:160的ActRIIA-Fc和TGFβRII$_{LONG}$-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ActRIIA-Fc:TGFβRII$_{LONG}$-Fc的异聚体复合体。

[1121] 反向静电方法

[1122] 在反向方法中,与上文描述的第一种方法相比,受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例30中以SEQ ID NO:151-152提供ActRIIA-Fc融合蛋白的备选多肽序列。

[1123] 实施例24中以SEQ ID NO:130-132提供TGFβRII$_{LONG}$-Fc融合蛋白的多肽序列和其编码核酸序列。

[1124] SEQ ID NO:152和SEQ ID NO:132的ActRIIA-Fc和TGFβRII$_{LONG}$-Fc融合蛋白分别可

共表达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:TGFβRII$_{LONG}$-Fc的变体异聚体复合体。

[1125] 疏水方法

[1126] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例14中以SEQ ID NO409-410提供ActRIIA-Fc融合蛋白的多肽序列。

[1127] TGFβRII$_{LONG}$-Fc融合多肽的互补形式(SEQ ID NO:461)如下：

```
  1  MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51  RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
101  TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
151  KEKKKPGETF FMCSCSSDEC NDNIIFSEEY NTSNPDTGGG THTCPPCPAP
201  ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
251  EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
301  EKTISKAKGQ PREPQVCTLP PSREEMTKNQ VSLSCAVKGF YPSDIAVEWE
351  SNGQPENNYK TTPPVLDSDG SFFLVSKLTV DKSRWQQGNV FSCSVMHEAL
401  HNHYTQKSLS LSPGK   (SEQ ID NO: 461)
```

[1128]

[1129] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:409-410的ActRIIA-Fc融合多肽形成异二聚体，可将4个氨基酸取代引入上文双下划线所示ActRIIB-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:461的氨基酸序列。

[1130] 成熟的TGFβRII$_{LONG}$-Fc融合蛋白序列(SEQ ID NO:462)如下，并可任选提供从C端脱去赖氨酸的序列。

```
  1  TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51  PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL
101  ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
151  IFSEEYNTSN PDTGGGTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
201  PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
251  TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVCTLPPSRE
301  EMTKNQVSLS CAVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
351  VSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
     (SEQ ID NO: 462)
```

[1131]

[1132] SEQ ID NO:410和SEQ ID NO:462的ActRIIA-Fc和TGFβRII$_{LONG}$-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:TGFβRII$_{LONG}$-Fc的变体异聚体复合体。

[1133] 反向疏水方法

[1134] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，按紧接的上文所述，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而，与上述方法相比，该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例30中以SEQ ID NO:451-452提供ActRIIA-Fc融合蛋白的备选多肽序列。

[1135] 实施例24中以SEQ ID NO:417-418提供TGFβRII$_{LONG}$-Fc融合蛋白的多肽序列。

[1136] SEQ ID NO:452和SEQ ID NO:418的ActRIIA-Fc和TGFβRII$_{LONG}$-Fc蛋白分别可共表

达并从CHO细胞系中纯化，以产生包含ActRIIA-Fc:TGFβRII$_{LONG}$-Fc的变体异聚体复合体。

[1137] 不同ActRIIA-Fc:TGFβRII$_{LONG}$-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1138] 实施例35.包含两种II型受体的其它异二聚体的产生

[1139] 可通过类似于描述于实施例30所述方法引导包含两种II型受体的胞外域的其它的异二聚体蛋白质复合体的形成。下表公开了这些变体异二聚体的氨基酸SEQ ID NO,对其完整性还包括已论述的包含两种II型受体的异二聚体(实施例30-34)。如实施例30-34中所公开的，各受体-Fc融合蛋白的C端赖氨酸可任选包括或删除。

[1140] 在每种情况下，可产生包含2种不同II型受体的胞外域(ECD)(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性异聚体复合体。在每种情况下，融合蛋白可共表达并从CHO细胞系中纯化，以产生变体异聚体蛋白质复合体。在每种情况下，不同异聚体复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1141] 表:“II型:II型受体异二聚体”

[1142]

II 型:II 型受体异二聚体的氨基酸 SEQ ID NO					
异聚体融合蛋白复合体	Fc 对类型	对应于 Fc 对的 ECD	受体-Fc 融合蛋白	氨基酸 SEQ ID NO	
				含前导序列	成熟
ActRIIA-Fc: ActRIIB-Fc	静电	A	ActRIIA-Fc	118	120
			ActRIIB-Fc	153	154
		B	ActRIIA-Fc	151	152
			ActRIIB-Fc	100	102
	疏水	A	ActRIIA-Fc	409	410
			ActRIIB-Fc	453	454
		B	ActRIIA-Fc	451	452
			ActRIIB-Fc	401	402
ActRIIA-Fc: BMPRII-Fc	静电	A	ActRIIA-Fc	118	120
			BMPRII-Fc	155	156
		B	ActRIIA-Fc	151	152
			BMPRII-Fc	121	123
	疏水	A	ActRIIA-Fc	409	410
			BMPRII-Fc	455	456
		B	ActRIIA-Fc	451	452
			BMPRII-Fc	411	412
ActRIIA-Fc: MISRII-Fc	静电	A	ActRIIA-Fc	118	120
			MISRII-Fc	161	162
		B	ActRIIA-Fc	151	152
			MISRII-Fc	133	135
	疏水	A	ActRIIA-Fc	409	410
			MISRII-Fc	457	458
		B	ActRIIA-Fc	451	452
			MISRII-Fc	419	420
ActRIIA-Fc:	静电	A	ActRIIA-Fc	118	120

[1143]

TGFβRII$_{SHORT}$-Fc			TGFβRII$_{SHORT}$-Fc	157	158
		B	ActRIIA-Fc	151	152
			TGFβRII$_{SHORT}$-Fc	127	129
	疏水	A	ActRIIA-Fc	409	410
			TGFβRII$_{SHORT}$-Fc	459	460
		B	ActRIIA-Fc	451	452
			TGFβRII$_{SHORT}$-Fc	415	416
ActRIIA-Fc:TGFβRII$_{LONG}$-Fc	静电	A	ActRIIA-Fc	118	120
			TGFβRII$_{LONG}$-Fc	159	160
		B	ActRIIA-Fc	151	152
			TGFβRII$_{LONG}$-Fc	130	132
	疏水	A	ActRIIA-Fc	409	410
			TGFβRII$_{LONG}$-Fc	461	462
		B	ActRIIA-Fc	451	452
			TGFβRII$_{LONG}$-Fc	417	418
ActRIIB-Fc:BMPRII-Fc	静电	A	ActRIIB-Fc	100	102
			BMPRII-Fc	155	156
		B	ActRIIB-Fc	153	154
			BMPRII-Fc	121	123
	疏水	A	ActRIIB-Fc	401	402
			BMPRII-Fc	455	456
		B	ActRIIB-Fc	453	454
			BMPRII-Fc	411	412
ActRIIB-Fc:MISRII-Fc	静电	A	ActRIIB-Fc	100	102
			MISRII-Fc	161	162
		B	ActRIIB-Fc	153	154
			MISRII-Fc	133	135
	疏水	A	ActRIIB-Fc	401	402
			MISRII-Fc	457	458
		B	ActRIIB-Fc	453	454
			MISRII-Fc	419	420
ActRIIB-Fc:TGF	静电	A	ActRIIB-Fc	100	102

[1144]

βRII_{SHORT}-Fc			TGFβRII_{SHORT}-Fc	157	158
		B	ActRIIB-Fc	153	154
			TGFβRII_{SHORT}-Fc	127	129
	疏水	A	ActRIIB-Fc	401	402
			TGFβRII_{SHORT}-Fc	459	460
		B	ActRIIB-Fc	453	454
			TGFβRII_{SHORT}-Fc	415	416
ActRIIB-Fc:TGFβRII_{LONG}-Fc	静电	A	ActRIIB-Fc	100	102
			TGFβRII_{LONG}-Fc	159	160
		B	ActRIIB-Fc	153	154
			TGFβRII_{LONG}-Fc	130	132
	疏水	A	ActRIIB-Fc	401	402
			TGFβRII_{LONG}-Fc	461	462
		B	ActRIIB-Fc	453	454
			TGFβRII_{LONG}-Fc	417	418
BMPRII-Fc:MISRII-Fc	静电	A	BMPRII-Fc	121	123
			MISRII-Fc	161	162
		B	BMPRII-Fc	155	156
			MISRII-Fc	133	135
	疏水	A	BMPRII-Fc	411	412
			MISRII-Fc	457	458
		B	BMPRII-Fc	455	456
			MISRII-Fc	419	420
BMPRII-Fc:TGFβRII_{SHORT}-Fc	静电	A	BMPRII-Fc	121	123
			TGFβRII_{SHORT}-Fc	157	158
		B	BMPRII-Fc	155	156
			TGFβRII_{SHORT}-Fc	127	129
	疏水	A	BMPRII-Fc	411	412
			TGFβRII_{SHORT}-Fc	459	460

[1145]

		B	BMPRII-Fc	455	456
			$TGF\beta RII_{SHORT}$-Fc	415	416
BMPRII-Fc:$TGF\beta RII_{LONG}$-Fc	静电	A	BMPRII-Fc	121	123
			$TGF\beta RII_{LONG}$-Fc	159	160
		B	BMPRII-Fc	155	156
			$TGF\beta RII_{LONG}$-Fc	130	132
	疏水	A	BMPRII-Fc	411	412
			$TGF\beta RII_{LONG}$-Fc	461	462
		B	BMPRII-Fc	455	456
			$TGF\beta RII_{LONG}$-Fc	417	418
MISRII-Fc:$TGF\beta RII_{SHORT}$-Fc	静电	A	MISRII-Fc	133	135
			$TGF\beta RII_{SHORT}$-Fc	157	158
		B	MISRII-Fc	161	162
			$TGF\beta RII_{SHORT}$-Fc	127	129
	疏水	A	MISRII-Fc	419	420
			$TGF\beta RII_{SHORT}$-Fc	459	460
		B	MISRII-Fc	457	458
			$TGF\beta RII_{SHORT}$-Fc	415	416
MISRII-Fc:$TGF\beta RII_{LONG}$-Fc	静电	A	MISRII-Fc	133	135
			$TGF\beta RII_{LONG}$-Fc	159	160
		B	MISRII-Fc	161	162
			$TGF\beta RII_{LONG}$-Fc	130	132
	疏水	A	MISRII-Fc	419	420
			$TGF\beta RII_{LONG}$-Fc	461	462
		B	MISRII-Fc	457	458
			$TGF\beta RII_{LONG}$-Fc	417	418
$TGF\beta RII_{SHORT}$-Fc:$TGF\beta RII_{LONG}$-Fc	静电	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			$TGF\beta RII_{LONG}$-Fc	159	160
		B	$TGF\beta RII_{SHORT}$-Fc	157	158

[1146]

			$TGF\beta RII_{LONG}$-Fc	130	132
	疏水	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			$TGF\beta RII_{LONG}$-Fc	461	462
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			$TGF\beta RII_{LONG}$-Fc	417	418

[1147] 实施例36.ALK1-Fc:ALK2-Fc异二聚体的产生

[1148] 可产生包含人ALK1和人ALK2的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ALK1-Fc:ALK2-Fc异聚体复合体。各个构建体分别被称为ALK1-Fc和ALK2-Fc融合蛋白。

[1149] 通过类似于实施例1所述方法可引导异聚体ALK1-Fc:ALK2-Fc的形成。

[1150] 静电方法

[1151] 在第一种方法中,实施例16中以SEQ ID NO:124-126提供ALK1-Fc融合蛋白的的多肽序列和其编码核酸序列。

[1152] 互补ALK2-Fc融合蛋白的多肽序列(SEQ ID NO:173)使用TPA前导序列,且列举如下:

[1153]

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
301  KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPG (SEQ ID NO: 173)
```

[1154] 前导序列和接头序列用下划线表示。为了促进ALK1-Fc:ALK2-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端添加赖氨酸的SEQ ID NO:173的氨基酸序列。

[1155] 成熟的ALK2-Fc融合蛋白序列(SEQ ID NO:174)如下,且可任选提供C端添加赖氨酸的序列。

```
   1  MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG QQCFSSLSIN DGFHVYQKGC
  51  FQVYEQGKMT CKTPPSPGQA VECCQGDWCN RNITAQLPTK GKSFPGTQNF
 101  HLETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR KEMTKNQVSL
 251  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF LYSKLTVDKS
 301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G    (SEQ ID NO: 174)
```

[1156]

[1157] SEQ ID NO:126和SEQ ID NO:174的ALK1-Fc和ALK2-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含的异聚体复合体ALK1-Fc:ALK2-Fc。

[1158] 反向静电方法

[1159] 在反向方法中,与上文描述的第一种方法相比,受体胞外域同时与Fc相互作用对的对立成员连接。在该实施例中,ALK1-Fc融合蛋白的备选多肽序列(SEQ ID NO:171)使用TPA前导序列,且列举如下。

```
   1  MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
  51  GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
 101  NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
 151  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
 201  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
 251  PQVYTLPPSR KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
 301  PVLKSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
 351  G         (SEQ ID NO: 171)
```

[1160]

[1161] 前导序列和接头序列用下划线表示。为了促进ALK1-Fc:ALK2-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端添加赖氨酸的SEQ ID NO:171的氨基酸序列。

[1162] 成熟的ALK1-Fc融合蛋白序列(SEQ ID NO:172)如下,且可任选提供C端添加赖氨酸的序列。

[1163]

```
   1  DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
  51  LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
 101  GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 151  DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 201  KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRKEMT KNQVSLTCLV
 251  KGFYPSDIAV EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ
 301  GNVFSCSVMH EALHNHYTQK SLSLSPG         (SEQ ID NO: 172)
```

[1164]

[1165] 实施例9中以SEQ ID NO:136-138提供ALK2-Fc融合蛋白的多肽序列和其编码核酸序列。

[1166] SEQ ID NO:172和SEQ ID NO:138的ALK1-Fc和ALK2-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK2-Fc的变体异聚体复合体。

[1167] 疏水方法

[1168] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中以SEQ ID NO:413-414提供ALK1-Fc融合蛋白的多肽序列。

[1169] ALK2-Fc融合多肽的互补形式(SEQ ID NO:465)如下：

[1170]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGAMEDEKP KVNPKLYMCV CEGLSCGNED
 51  HCEGQQCFSS LSINDGFHVY QKGCFQVYEQ GKMTCKTPPS PGQAVECCQG
101  DWCNRNITAQ LPTKGKSFPG TQNFHLETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW ESNGQPENNY
301  KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPGK        (SEQ ID NO: 465)
```

[1171] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:413-414的ALK1-Fc融合多肽形成异二聚体，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示ALK2-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:465的氨基酸序列。

[1172] 成熟的ALK2-Fc融合蛋白序列(SEQ ID NO:466)如下，并可任选提供从C端脱去赖氨酸的序列。

[1173]
```
  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 PQVYTLPPCR EEMTKNQVSL
251  WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 466)
```

[1174] SEQ ID NO:414和SEQ ID NO:466的ALK1-Fc和ALK2-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK2-Fc的变体异聚体复合体。

[1175] 反向疏水方法

[1176] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，按紧接的上文所述，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而，与上述方法相比，该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，ALK1-Fc融合蛋白的备选多肽序列(SEQ ID NO:463)使用TPA前导序列，且列举如下。

[1177]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGADPVKPS RGPLVTCTCE SPHCKGPTCR
 51   GAWCTVVLVR EEGRHPQEHR GCGNLHRELC RGRPTEFVNH YCCDSHLCNH
101   NVSLVLEATQ PPSEQPGTDG QLATGGGTHT CPPCPAPELL GGPSVFLFPP
151   KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
201   YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
251   PQVYTLPPCR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP
301   PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
351   GK       (SEQ ID NO: 463)
```

[1178] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO421-422的ALK2-Fc融合多肽形成异二聚体，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示ALK1-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:463的氨基酸序列。

[1179] 成熟的ALK1-Fc融合蛋白序列(SEQ ID NO:464)如下，并可任选提供从C端脱去赖氨酸的序列。

[1180]

```
  1   DPVKPSRGPL VTCTCESPHC KGPTCRGAWC TVVLVREEGR HPQEHRGCGN
 51   LHRELCRGRP TEFVNHYCCD SHLCNHNVSL VLEATQPPSE QPGTDGQLAT
101   GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
151   DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
201   KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV
251   KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
301   GNVFSCSVMH EALHNHYTQK SLSLSPGK       (SEQ ID NO: 464)
```

[1181]

[1182] 实施例9中以SEQ ID NO:421-422提供ALK2-Fc融合蛋白的多肽序列。

[1183] SEQ ID NO:464和SEQ ID NO:422的ALK1-Fc和ALK2-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK2-Fc的变体异聚体复合体。

[1184] 不同ALK1-Fc:ALK2-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1185] 实施例37.ALK1-Fc:ALK3-Fc异二聚体的产生

[1186] 可产生包含人ALK1和人ALK3的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ALK1-Fc:ALK3-Fc异聚体复合体。各个构建体分别被称为ALK1-Fc和ALK3-Fc融合蛋白。

[1187] 可采用类似于实施例36的方法引导异聚体ALK1-Fc:ALK3-Fc的形成。

[1188] 静电方法

[1189] 在第一种方法中，实施例16中以SEQ ID NO:124-126提供ALK1-Fc融合蛋白的多肽序列和其编码核酸序列。

[1190] 互补ALK3-Fc融合蛋白的多肽序列(SEQ ID NO:175)使用TPA前导序列，且列举如下：

[1191]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51   LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
101   CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
151   SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
```

[1192]

```
201   VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251   GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR KEMTKNQVSL
301   TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF LYSKLTVDKS
351   RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G    (SEQ ID NO: 175)
```

[1193] 前导序列和接头序列用下划线表示。为了促进ALK1-Fc:ALK3-Fc异二聚体而非可能的同二聚体复合体的任一个的形成，可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端添加赖氨酸的SEQ ID NO:175的氨基酸序列。

[1194] 成熟的ALK3-Fc融合蛋白序列(SEQ ID NO:176)如下，且可任选提供C端添加赖氨酸的序列。

[1195]

```
  1   GAQNLDSMLH GTGMKSDSDQ KKSENGVTLA PEDTLPFLKC YCSGHCPDDA
 51   INNTCITNGH CFAIIEEDDQ GETTLASGCM KYEGSDFQCK DSPKAQLRRT
101   IECCRTNLCN QYLQPTLPPV VIGPFFDGSI RTGGGTHTCP PCPAPELLGG
151   PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201   KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251   KAKGQPREPQ VYTLPPSRKE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301   ENNYKTTPPV LKSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351   QKSLSLSPG              (SEQ ID NO: 176)
```

[1196] SEQ ID NO:126和SEQ ID NO:176的ALK1-Fc和ALK3-Fc融合蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK3-Fc的异聚体复合体。

[1197] 反向静电方法

[1198] 在反向方法中，与上文描述的第一种方法相比，受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例36中以SEQ ID NO:171-172提供ALK1-Fc融合蛋白的备选多肽序列。

[1199] ALK3-Fc融合蛋白的多肽序列和其编码核酸序列在实施例4中以SEQ ID NO:115-117提供。

[1200] SEQ ID NO:172和SEQ ID NO:117的ALK1-Fc和ALK3-Fc融合蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK3-Fc的变体异聚体复合体。

[1201] 疏水方法

[1202] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中以SEQ ID NO413-414提供ALK1-Fc融合蛋白的多肽序列。

[1203] ALK3-Fc融合多肽的互补形式(SEQ ID NO:467)如下：

[1204]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51   LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
101   CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
151   SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201   VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251   GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR EEMTKNQVSL
301   WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
351   RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK  (SEQ ID NO: 467)
```

[1205] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:413-414的ALK1-Fc融合多肽形成异二聚体，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示ALK3-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:467的氨基酸序列。

[1206] 成熟的ALK3-Fc融合蛋白序列(SEQ ID NO:468)如下，并可任选提供从C端脱去赖氨酸的序列。

[1207]

```
  1   GAQNLDSMLH GTGMKSDSDQ KKSENGVTLA PEDTLPFLKC YCSGHCPDDA
 51   INNTCITNGH CFAIIEEDDQ GETTLASGCM KYEGSDFQCK DSPKAQLRRT
101   IECCRTNLCN QYLQPTLPPV VIGPFFDGSI RTGGGTHTCP PCPAPELLGG
151   PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201   KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251   KAKGQPREPQ VYTLPPCREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
301   ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351   QKSLSLSPGK            (SEQ ID NO: 468)
```

[1208] SEQ ID NO:414和SEQ ID NO:468的ALK1-Fc和ALK3-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK3-Fc的变体异聚体复合体。

[1209] 反向疏水方法

[1210] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，按紧接的上文所述，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而，与上述方法相比，该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例36中以SEQ ID NO:463-464提供ALK1-Fc融合蛋白的备选多肽序列。

[1211] 实施例4中以SEQ ID NO:407-408提供ALK3-Fc融合蛋白的多肽序列。

[1212] SEQ ID NO:464和SEQ ID NO:408的ALK1-Fc和ALK3-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK3-Fc的变体异聚体复合体。

[1213] 不同ALK1-Fc:ALK3-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1214] 实施例38.ALK1-Fc:ALK4-Fc异二聚体的产生

[1215] 可产生包含人ALK1和人ALK4的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ALK1-Fc:ALK4-Fc异聚体复合体。各个构建体分别被称为ALK1-Fc和ALK4-Fc融合蛋白。

[1216] 可采用类似于实施例36的方法引导异聚体ALK1-Fc:ALK4-Fc的形成。

[1217] 静电方法

[1218] 在第一种方法中,实施例16中以SEQ ID NO:124-126提供ALK1-Fc融合蛋白的多肽序列和其编码核酸序列。

[1219] 互补ALK4-Fc融合蛋白的多肽序列(SEQ ID NO:177)使用TPA前导序列,且列举如下:

[1220]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 51  GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101  YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
```

[1221]
```
251  QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
301  KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPG      (SEQ ID NO: 177)
```

[1222] 前导序列和接头序列用下划线表示。为了促进ALK1-Fc:ALK4-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端添加赖氨酸的SEQ ID NO:177的氨基酸序列。

[1223] 成熟的ALK4-Fc融合蛋白序列(SEQ ID NO:178)如下,且可任选提供C端添加赖氨酸的序列。

[1224]
```
  1  SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
 51  ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101  PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR KEMTKNQVSL
251  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF LYSKLTVDKS
301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G    (SEQ ID NO: 178)
```

[1225] SEQ ID NO:126和SEQ ID NO:178的ALK1-Fc和ALK4-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK4-Fc的异聚体复合体。

[1226] 反向静电方法

[1227] 在反向方法中,与上文描述的第一种方法相比,受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例36中以SEQ ID NO:171-172提供ALK1-Fc融合蛋白的备选多肽序列。

[1228] 实施例1中以SEQ ID NO:104-106提供ALK4-Fc融合蛋白的多肽序列和其编码核酸序列。

[1229] SEQ ID NO:172和SEQ ID NO:106的ALK1-Fc和ALK4-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK4-Fc的变体异聚体复合体。

[1230] 疏水方法

[1231] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,改变Fc结构

域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中以SEQ ID NO413-414提供ALK1-Fc融合蛋白的多肽序列。

[1232] ALK4-Fc融合多肽的互补形式(SEQ ID NO:469)如下：

[1233]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 51   GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101   YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151   LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201   REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251   QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW ESNGQPENNY
301   KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351   SLSPGK    (SEQ ID NO: 469)
```

[1234] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:413-414的ALK1-Fc融合多肽形成异二聚体，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示ALK4-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:469的氨基酸序列。

[1235] 成熟的ALK4-Fc融合蛋白序列(SEQ ID NO:470)如下，并可任选提供从C端脱去赖氨酸的序列。

[1236]

```
  1   SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
 51   ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101   PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151   VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201   GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR EEMTKNQVSL
251   WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
301   RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK    (SEQ ID NO: 470)
```

[1237] SEQ ID NO:414和SEQ ID NO:470的ALK1-Fc和ALK4-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK4-Fc的变体异聚体复合体。

[1238] 反向疏水方法

[1239] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，按紧接的上文所述，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而，与上述方法相比，该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例36中以SEQ ID NO:463-464提供ALK1-Fc融合蛋白的备选多肽序列。

[1240] 实施例1中以SEQ ID NO:403-404提供ALK4-Fc融合蛋白的多肽序列。

[1241] SEQ ID NO:464和SEQ ID NO:404的ALK1-Fc和ALK4-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK4-Fc的变体异聚体复合体。

[1242] 不同ALK1-Fc:ALK4-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1243] 实施例39.ALK1-Fc:ALK5-Fc异二聚体的产生

[1244] 可产生包含人ALK1和人ALK5的胞外域(其各自与具有位于胞外域和Fc结构域之间

的接头的Fc结构域融合)的可溶性ALK1-Fc:ALK5-Fc异聚体复合体。各个构建体分别被称为ALK1-Fc和ALK5-Fc融合蛋白。

[1245] 可采用类似于实施例36的方法引导异聚体ALK1-Fc:ALK5-Fc的形成。

[1246] 静电方法

[1247] 在第一种方法中,实施例16中以SEQ ID NO:124-126提供ALK1-Fc融合蛋白的多肽序列和其编码核酸序列。

[1248] 互补ALK5-Fc融合蛋白的多肽序列(SEQ ID NO:179)使用TPA前导序列,且列举如下:

[1249]

```
  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
```

[1250]

```
251   EPQVYTLPPS RKEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT
301   PPVLKSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
351   PG    (SEQ ID NO: 179)
```

[1251] 前导序列和接头序列用下划线表示。为了促进的形成ALK1-Fc:ALK5-Fc异二聚体而非可能的同二聚体复合体的任一个,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端添加赖氨酸的SEQ ID NO:179的氨基酸序列。

[1252] 成熟的ALK5-Fc融合蛋白序列(SEQ ID NO:180)如下,且可任选提供C端添加赖氨酸的序列。

[1253]

```
  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 YTLPPSRKEM TKNQVSLTCL
251   VKGFYPSDIA VEWESNGQPE NNYKTTPPVL KSDGSFFLYS KLTVDKSRWQ
301   QGNVFSCSVM HEALHNHYTQ KSLSLSPG    (SEQ ID NO: 180)
```

[1254] SEQ ID NO:126和SEQ ID NO:180的ALK1-Fc和ALK5-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK5-Fc的异聚体复合体。

[1255] 反向静电方法

[1256] 在反向方法中,与上文描述的第一种方法相比,受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例36中以SEQ ID NO:171-172提供ALK1-Fc融合蛋白的备选多肽序列。

[1257] 实施例11中以SEQ ID NO:139-141提供ALK5-Fc融合蛋白的多肽序列和其编码核酸序列。

[1258] SEQ ID NO:172和SEQ ID NO:141的ALK1-Fc和ALK5-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK5-Fc的变体异聚体复合体。

[1259] 疏水方法

[1260] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中以SEQ ID NO413-414提供ALK1-Fc融合蛋白的多肽序列。

[1261] ALK5-Fc融合多肽的互补形式(SEQ ID NO:471)如下：

[1262]
```
  1   MDAMKRGLCC VLLLCGAVFV SPGAALLPGA TALQCFCHLC TKDNFTCVTD
 51   GLCFVSVTET TDKVIHNSMC IAEIDLIPRD RPFVCAPSSK TGSVTTTYCC
101   NQDHCNKIEL PTTVKSSPGL GPVETGGGTH TCPPCPAPEL LGGPSVFLFP
151   PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
201   QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
251   EPQVYTLPPC REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
301   PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
351   PGK       (SEQ ID NO: 471)
```

[1263] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:413-414的ALK1-Fc融合多肽形成异二聚体，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示ALK5-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:471的氨基酸序列。

[1264] 成熟的ALK5-Fc融合蛋白序列(SEQ ID NO:472)如下，并可任选提供从C端脱去赖氨酸的序列。

[1265]
```
 1A   LLPGATALQ 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 YTLPPCREEM TKNQVSLWCL
251   VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ
301   QGNVFSCSVM HEALHNHYTQ KSLSLSPGK       (SEQ ID NO: 472)
```

[1266] 上文双下划线所示ALK5-Fc融合多肽的Fc结构域中的ALK1-Fc和ALK5-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK5-Fc的变体异聚体复合体。

[1267] 反向疏水方法

[1268] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，按紧接的上文所述，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而，与上述方法相比，该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例36中以SEQ ID NO:463-464提供ALK1-Fc融合蛋白的备选多肽序列。

[1269] 实施例16中以SEQ ID NO:423-424提供ALK5-Fc融合蛋白的多肽序列。

[1270] SEQ ID NO:464和SEQ ID NO:424的ALK1-Fc和ALK5-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK5-Fc的变体异聚体复合体。

[1271] 不同ALK1-Fc:ALK5-Fc复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析

法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1272] 实施例40.ALK1-Fc:ALK6-Fc异二聚体的产生

[1273] 可产生包含人ALK1和人ALK6的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ALK1-Fc:ALK6-Fc异聚体复合体。各个构建体分别被称为ALK1-Fc和ALK6-Fc融合蛋白。

[1274] 可采用类似于实施例36的方法引导异聚体ALK1-Fc:ALK6-Fc的形成。

[1275] 静电方法

[1276] 在第一种方法中,实施例16中以SEQ ID NO:124-126提供ALK1-Fc融合蛋白的多肽序列和其编码核酸序列。

[1277] 互补ALK6-Fc融合蛋白的多肽序列(SEQ ID NO:181)使用TPA前导序列,且列举如下:

[1278]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51   EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLESGDF QCRDTPIPHQ
101   RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151   PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201   VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251   IEKTISKAKG QPREPQVYTL PPSRKEMTKN QVSLTCLVKG FYPSDIAVEW
301   ESNGQPENNY KTTPPVLKSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351   LHNHYTQKSL SLSPG      (SEQ ID NO: 181)
```

[1279]

[1280] 前导序列和接头序列用下划线表示。为了促进ALK1-Fc:ALK6-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端添加赖氨酸的SEQ ID NO:181的氨基酸序列。

[1281] 成熟的ALK6-Fc融合蛋白序列(SEQ ID NO:182)如下,且可任选提供C端添加赖氨酸的序列。

[1282]

```
  1   KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
 51   LPVVTSGCLG LESGDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101   KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151   TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201   LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251   KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF
301   LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
      (SEQ ID NO: 182)
```

[1283] SEQ ID NO:126和SEQ ID NO:182的ALK1-Fc和ALK6-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK6-Fc的异聚体复合体。

[1284] 反向静电方法

[1285] 在反向方法中,与上文描述的第一种方法相比,受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例36中以SEQ ID NO:171-172提供ALK1-Fc融合蛋白的备选多肽序列。

[1286] 实施例13中以SEQ ID NO:142-144提供ALK6-Fc融合蛋白的多肽序列和其编码核酸序列。

[1287] SEQ ID NO:172和SEQ ID NO:144的ALK1-Fc和ALK6-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK6-Fc的变体异聚体复合体。

[1288] 疏水方法

[1289] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中以SEQ ID NO413-414提供ALK1-Fc融合蛋白的多肽序列。

[1290] ALK6-Fc融合多肽的互补形式(SEQ ID NO:473)如下:

[1291]
```
  1   MDAMKRGLCC VLLLCGAVFV SPGAKKEDGE STAPTPRPKV LRCKCHHHCP
 51   EDSVNNICST DGYCFTMIEE DDSGLPVVTS GCLGLESGDF QCRDTPIPHQ
101   RRSIECCTER NECNKDLHPT LPPLKNRDFV DGPIHHRTGG GTHTCPPCPA
151   PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
201   VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP
251   IEKTISKAKG QPREPQVYTL PPCREEMTKN QVSLWCLVKG FYPSDIAVEW
301   ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
351   LHNHYTQKSL SLSPGK        (SEQ ID NO: 473)
```

[1292] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:413-414的ALK1-Fc融合多肽形成异二聚体,可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示ALK6-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:473的氨基酸序列。

[1293] 成熟的ALK6-Fc融合蛋白序列(SEQ ID NO:474)如下,并可任选提供从C端脱去赖氨酸的序列。

[1294]
```
  1   KKEDGESTAP TPRPKVLRCK CHHHCPEDSV NNICSTDGYC FTMIEEDDSG
 51   LPVVTSGCLG LESGDFQCRD TPIPHQRRSI ECCTERNECN KDLHPTLPPL
101   KNRDFVDGPI HHRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151   TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201   LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR
251   EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301   LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
      (SEQ ID NO: 474)
```

[1295] SEQ ID NO:414和SEQ ID NO:474的ALK1-Fc和ALK6-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK6-Fc的变体异聚体复合体。

[1296] 反向疏水方法

[1297] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中,按紧接的上文所述,改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而,与上述方法相比,该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中,实施例36中以SEQ ID NO:463-464提供ALK1-Fc融合蛋白的备选多肽序列。

[1298] 实施例13中以SEQ ID NO:425-426提供ALK6-Fc融合蛋白的多肽序列。

[1299] SEQ ID NO:464和SEQ ID NO:426的ALK1-Fc和ALK6-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK6-Fc的变体异聚体复合体。

[1300] 不同ALK1-Fc:ALK6-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1301] 实施例41.ALK1-Fc:ALK7-Fc异二聚体的产生

[1302] 可产生包含人ALK1和人ALK7的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性ALK1-Fc:ALK7-Fc异聚体复合体。各个构建体分别被称为ALK1-Fc和ALK7-Fc融合蛋白。

[1303] 可采用类似于实施例36的方法引导异聚体ALK1-Fc:ALK7-Fc的形成。

[1304] 静电方法

[1305] 在第一种方法中,实施例16中以SEQ ID NO:124-126提供ALK1-Fc融合蛋白的多肽序列和其编码核酸序列。

[1306] 互补ALK7-Fc融合蛋白的多肽序列(SEQ ID NO:183)使用TPA前导序列,且列举如下:

[1307]

```
  1   MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51   MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101   TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151   TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201   LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251   KEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLKSDGSFF
301   LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
```

[1308] (SEQ ID NO: 183)

[1309] 前导序列和接头序列用下划线表示。为了促进ALK1-Fc:ALK7-Fc异二聚体而非可能的同二聚体复合体的任一个的形成,可将2个氨基酸取代(将酸性氨基酸用赖氨酸置换)引入上文双下划线所示融合蛋白的Fc结构域中。可任选提供C端添加赖氨酸的SEQ ID NO:183的氨基酸序列。

[1310] 预期成熟的ALK7-Fc融合蛋白序列如下(SEQ ID NO:184),且可任选提供C端添加赖氨酸的序列。

[1311]

```
  1   GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51   CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101   PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNG关键KCKVSNKALP
201   APIEKTISKA KGQPREPQVY TLPPSRKEMT KNQVSLTCLV KGFYPSDIAV
251   EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
301   EALHNHYTQK SLSLSPG          (SEQ ID NO: 184)
```

[1312] SEQ ID NO:126和SEQ ID NO:184的ALK1-Fc和ALK7-Fc融合蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK7-Fc的异聚体复合体。

[1313] 反向静电方法

[1314] 在反向方法中，与上文描述的第一种方法相比，受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例36中以SEQ ID NO:171-172提供ALK1-Fc融合蛋白的备选多肽序列。

[1315] 实施例7中以SEQ ID NO:112-114提供ALK7-Fc融合蛋白的多肽序列和其编码核酸序列。

[1316] SEQ ID NO:172和SEQ ID NO:114的ALK1-Fc和ALK7-Fc融合蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK7-Fc的变体异聚体复合体。

[1317] 疏水方法

[1318] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。实施例16中以SEQ ID NO413-414提供ALK1-Fc融合蛋白的多肽序列。

[1319] ALK7-Fc融合多肽的互补形式(SEQ ID NO:475)如下：

[1320]
```
  1   MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51   MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101   TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151   TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201   LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPCR
251   EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301   LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
      (SEQ ID NO: 475)
```

[1321] 前导序列和接头序列用下划线表示。为了引导用SEQ ID NO:413-414的ALK1-Fc融合多肽形成异二聚体，可将2个氨基酸取代(将丝氨酸用半胱氨酸置换并将苏氨酸用色氨酸置换)引入上文双下划线所示ALK7-Fc融合多肽的Fc结构域中。可任选提供从C端脱去赖氨酸的SEQ ID NO:475的氨基酸序列。

[1322] 预期成熟的ALK7-Fc融合蛋白序列(SEQ ID NO:476)如下，并可任选提供从C端脱去赖氨酸的序列。

[1323]
```
  1   GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51   CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101   PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNG关键KCKVSNKALP
201   APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPSDIAV
251   EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
301   EALHNHYTQK SLSLSPGK        (SEQ ID NO: 476)
```

[1324] SEQ ID NO:414和SEQ ID NO:476的ALK1-Fc和ALK7-Fc蛋白分别可共表达并从CHO细胞系中纯化，以产生包含ALK1-Fc:ALK7-Fc的变体异聚体复合体。

[1325] 反向疏水方法

[1326] 在使用不对称Fc融合蛋白促进异多聚体复合体形成的另一种方法中，按紧接的上文所述，改变Fc结构域以引入互补疏水相互作用和其它的分子间二硫键。然而，与上述方法相比，该方法中的受体胞外域与Fc相互作用对的对立成员连接。在该实施例中，实施例36中

以SEQ ID NO:463-464提供ALK1-Fc融合蛋白的备选多肽序列。

[1327] 实施例7中以SEQ ID NO:405-406提供ALK7-Fc融合蛋白的多肽序列。

[1328] SEQ ID NO:464和SEQ ID NO:406的ALK1-Fc和ALK7-Fc蛋白分别可共表达并从CHO细胞系中纯化,以产生包含ALK1-Fc:ALK7-Fc的变体异聚体复合体。

[1329] 不同ALK1-Fc:ALK7-Fc复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1330] 实施例42.包含两种I型受体的其它异二聚体的产生

[1331] 可通过类似于实施例36所述方法引导包含两种I型受体的胞外域的其它异二聚体蛋白质复合体的形成。下表公开了这些变体异二聚体的氨基酸SEQ ID NO,出于完整性还包括已论述的包含两种I型受体的异二聚体(实施例36-41)。如实施例36-41中公开的,各受体-Fc融合蛋白的C端赖氨酸可任选包括或删除。

[1332] 在每种情况下,可产生包含2种不同I型受体的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性异聚体复合体。在每种情况下,融合蛋白可共表达并从CHO细胞系中纯化,以产生变体异聚体蛋白质复合体。在每种情况下,不同异聚体蛋白质复合体的纯化可通过一系列柱层析法步骤实现,包括例如以下任何顺序的三种或更多种:A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1333] 表:“I型:I型受体异二聚体”

[1334]

I型:I型受体异二聚体的氨基酸 SEQ ID NO

[1335]

异聚体融合蛋白复合体	Fc 对类型	对应于 Fc 对的 ECD	受体-Fc 融合蛋白	氨基酸 SEQ ID NO	
				含前导序列	成熟的
ALK1-Fc: ALK2-Fc	静电	A	ALK1-Fc	124	126
			ALK2-Fc	173	174
		B	ALK1-Fc	171	172
			ALK2-Fc	136	138
	疏水	A	ALK1-Fc	413	414
			ALK2-Fc	465	466
		B	ALK1-Fc	463	464
			ALK2-Fc	421	422
ALK1-Fc: ALK3-Fc	静电	A	ALK1-Fc	124	126
			ALK3-Fc	175	176
		B	ALK1-Fc	171	172
			ALK3-Fc	115	117
	疏水	A	ALK1-Fc	413	414
			ALK3-Fc	467	468
		B	ALK1-Fc	463	464
			ALK3-Fc	407	408
ALK1-Fc: ALK4-Fc	静电	A	ALK1-Fc	124	126
			ALK4-Fc	177	178
		B	ALK1-Fc	171	172
			ALK4-Fc	104	106
	疏水	A	ALK1-Fc	413	414
			ALK4-Fc	469	470
		B	ALK1-Fc	463	464
			ALK4-Fc	403	404
ALK1-Fc: ALK5-Fc	静电	A	ALK1-Fc	124	126
			ALK5-Fc	179	180
		B	ALK1-Fc	171	172
			ALK5-Fc	139	141
	疏水	A	ALK1-Fc	413	414
			ALK5-Fc	471	472

[1336]

		B	ALK1-Fc	463	464
			ALK5-Fc	423	424
ALK1-Fc: ALK6-Fc	静电	A	ALK1-Fc	124	126
			ALK6-Fc	181	182
		B	ALK1-Fc	171	172
			ALK6-Fc	142	144
	疏水	A	ALK1-Fc	413	414
			ALK6-Fc	473	474
		B	ALK1-Fc	463	464
			ALK6-Fc	425	426
ALK1-Fc: ALK7-Fc	静电	A	ALK1-Fc	124	126
			ALK7-Fc	183	184
		B	ALK1-Fc	171	172
			ALK7-Fc	112	114
	疏水	A	ALK1-Fc	413	414
			ALK7-Fc	475	476
		B	ALK1-Fc	463	464
			ALK7-Fc	405	406
ALK2-Fc: ALK3-Fc	静电	A	ALK2-Fc	136	138
			ALK3-Fc	175	176
		B	ALK2-Fc	173	174
			ALK3-Fc	115	117
	疏水	A	ALK2-Fc	421	422
			ALK3-Fc	467	468
		B	ALK2-Fc	465	466
			ALK3-Fc	407	408
ALK2-Fc: ALK4-Fc	静电	A	ALK2-Fc	136	138
			ALK4-Fc	177	178
		B	ALK2-Fc	173	174
			ALK4-Fc	104	106
	疏水	A	ALK2-Fc	421	422
			ALK4-Fc	469	470
		B	ALK2-Fc	465	466
			ALK4-Fc	403	404
ALK2-Fc: ALK5-Fc	静电	A	ALK2-Fc	136	138
			ALK5-Fc	179	180

[1337]

		B	ALK2-Fc	173	174
			ALK5-Fc	139	141
	疏水	A	ALK2-Fc	421	422
			ALK5-Fc	471	472
		B	ALK2-Fc	465	466
			ALK5-Fc	423	424
ALK2-Fc: ALK6-Fc	静电	A	ALK2-Fc	136	138
			ALK6-Fc	181	182
		B	ALK2-Fc	173	174
			ALK6-Fc	142	144
	疏水	A	ALK2-Fc	421	422
			ALK6-Fc	473	474
		B	ALK2-Fc	465	466
			ALK6-Fc	425	426
ALK2-Fc: ALK7-Fc	静电	A	ALK2-Fc	136	138
			ALK7-Fc	183	184
		B	ALK2-Fc	173	174
			ALK7-Fc	112	114
	疏水	A	ALK2-Fc	421	422
			ALK7-Fc	475	476
		B	ALK2-Fc	465	466
			ALK7-Fc	405	406
ALK3-Fc: ALK4-Fc	静电	A	ALK3-Fc	115	117
			ALK4-Fc	177	178
		B	ALK3-Fc	175	176
			ALK4-Fc	104	106
	疏水	A	ALK3-Fc	407	408
			ALK4-Fc	469	470
		B	ALK3-Fc	467	468
			ALK4-Fc	403	404
ALK3-Fc: ALK5-Fc	静电	A	ALK3-Fc	115	117
			ALK5-Fc	179	180
		B	ALK3-Fc	175	176
			ALK5-Fc	139	141
	疏水	A	ALK3-Fc	407	408
			ALK5-Fc	471	472

[1338]

		B	ALK3-Fc	467	468
			ALK5-Fc	423	424
ALK3-Fc: ALK6-Fc	静电	A	ALK3-Fc	115	117
			ALK6-Fc	181	182
		B	ALK3-Fc	175	176
			ALK6-Fc	142	144
	疏水	A	ALK3-Fc	407	408
			ALK6-Fc	473	474
		B	ALK3-Fc	467	468
			ALK6-Fc	425	426
ALK3-Fc: ALK7-Fc	静电	A	ALK3-Fc	115	117
			ALK7-Fc	183	184
		B	ALK3-Fc	175	176
			ALK7-Fc	112	114
	疏水	A	ALK3-Fc	407	408
			ALK7-Fc	475	476
		B	ALK3-Fc	467	468
			ALK7-Fc	405	406
ALK4-Fc: ALK5-Fc	静电	A	ALK4-Fc	104	106
			ALK5-Fc	179	180
		B	ALK4-Fc	177	178
			ALK5-Fc	139	141
	疏水	A	ALK4-Fc	403	404
			ALK5-Fc	471	472
		B	ALK4-Fc	469	470
			ALK5-Fc	423	424
ALK4-Fc: ALK6-Fc	静电	A	ALK4-Fc	104	106
			ALK6-Fc	181	182
		B	ALK4-Fc	177	178
			ALK6-Fc	142	144
	疏水	A	ALK4-Fc	403	404
			ALK6-Fc	473	474
		B	ALK4-Fc	469	470
			ALK6-Fc	425	426
ALK4-Fc: ALK7-Fc	静电	A	ALK4-Fc	104	106
			ALK7-Fc	183	184

[1339]

		B	ALK4-Fc	177	178
			ALK7-Fc	112	114
	疏水	A	ALK4-Fc	403	404
			ALK7-Fc	475	476
		B	ALK4-Fc	469	470
			ALK7-Fc	405	406
ALK5-Fc: ALK6-Fc	静电	A	ALK5-Fc	139	141
			ALK6-Fc	181	182
		B	ALK5-Fc	179	180
			ALK6-Fc	142	144
	疏水	A	ALK5-Fc	423	424
			ALK6-Fc	473	474
		B	ALK5-Fc	471	472
			ALK6-Fc	425	426
ALK5-Fc: ALK7-Fc	静电	A	ALK5-Fc	139	141
			ALK7-Fc	183	184
		B	ALK5-Fc	179	180
			ALK7-Fc	112	114
	疏水	A	ALK5-Fc	423	424
			ALK7-Fc	475	476
		B	ALK5-Fc	471	472
			ALK7-Fc	405	406
ALK6-Fc: ALK7-Fc	静电	A	ALK6-Fc	142	144
			ALK7-Fc	183	184
		B	ALK6-Fc	181	182
			ALK7-Fc	112	114
	疏水	A	ALK6-Fc	425	426
			ALK7-Fc	475	476
		B	ALK6-Fc	473	474
			ALK7-Fc	405	406

[1340] 实施例43.包含I型受体和II型受体的其它异二聚体的产生

[1341] 可通过类似于实施例1所述方法引导包含I型受体和II型受体的胞外域的其它异二聚体蛋白质复合体的形成。下表公开了这些变体异二聚体的氨基酸SEQ ID NO,出于完整性还包括已论述的包含I型受体和II型受体的异二聚体(实施例1-29)。如实施例1-29中公开的,各受体-Fc融合蛋白的C端赖氨酸可任选包括或删除。

[1342] 在每种情况下,可产生包含I型受体和II型受体的胞外域(其各自与具有位于胞外域和Fc结构域之间的接头的Fc结构域融合)的可溶性异聚体复合体。在每种情况下,融合蛋

白可共表达并从CHO细胞系中纯化，以产生变体异聚体蛋白质复合体。在每种情况下，不同异聚体蛋白质复合体的纯化可通过一系列柱层析法步骤实现，包括例如以下任何顺序的三种或更多种：A蛋白层析法、Q琼脂糖凝胶层析法、苯基琼脂糖凝胶层析法、大小排阻层析法和阳离子交换层析法。纯化可用病毒过滤和缓冲液交换完成。

[1343] 表："I型:II型受体异二聚体"

[1344]

I型:II型受体异二聚体的氨基酸 SEQ ID NO					
异聚体融合蛋白复合体	Fc对类型	对应于Fc对的ECD	受体-Fc融合蛋白	氨基酸 SEQ ID NO	
				含前导序列	成熟的
ActRIIA-Fc: ALK1-Fc	静电	A	ActRIIA-Fc	118	120
			ALK1-Fc	124	126
		B	ActRIIA-Fc	151	152
			ALK1-Fc	171	172
	疏水	A	ActRIIA-Fc	409	410
			ALK1-Fc	413	414
		B	ActRIIA-Fc	451	452
			ALK1-Fc	463	464
ActRIIA-Fc: ALK2-Fc	静电	A	ActRIIA-Fc	118	120
			ALK2-Fc	136	138
		B	ActRIIA-Fc	151	152
			ALK2-Fc	173	174
	疏水	A	ActRIIA-Fc	409	410
			ALK2-Fc	421	422
		B	ActRIIA-Fc	451	452
			ALK2-Fc	465	466

[1345]

ActRIIA-Fc: ALK3-Fc	静电	A	ActRIIA-Fc	118	120
			ALK3-Fc	115	117
		B	ActRIIA-Fc	151	152
			ALK3-Fc	175	176
	疏水	A	ActRIIA-Fc	409	410
			ALK3-Fc	407	408
		B	ActRIIA-Fc	451	452
			ALK3-Fc	467	468
ActRIIA-Fc: ALK4-Fc	静电	A	ActRIIA-Fc	118	120
			ALK4-Fc	104	106
		B	ActRIIA-Fc	151	152
			ALK4-Fc	177	178
	疏水	A	ActRIIA-Fc	409	410
			ALK4-Fc	403	404
		B	ActRIIA-Fc	451	452
			ALK4-Fc	469	470
ActRIIA-Fc: ALK5-Fc	静电	A	ActRIIA-Fc	118	120
			ALK5-Fc	139	141
		B	ActRIIA-Fc	151	152
			ALK5-Fc	179	180
	疏水	A	ActRIIA-Fc	409	410
			ALK5-Fc	423	424
		B	ActRIIA-Fc	451	452
			ALK5-Fc	471	472
ActRIIA-Fc: ALK6-Fc	静电	A	ActRIIA-Fc	118	120
			ALK6-Fc	142	144
		B	ActRIIA-Fc	151	152
			ALK6-Fc	181	182
	疏水	A	ActRIIA-Fc	409	410
			ALK6-Fc	425	426
		B	ActRIIA-Fc	451	452
			ALK6-Fc	473	474
ActRIIA-Fc: ALK7-Fc	静电	A	ActRIIA-Fc	118	120
			ALK7-Fc	112	114
		B	ActRIIA-Fc	151	152
			ALK7-Fc	183	184

[1346]

	疏水	A	ActRIIA-Fc	409	410
			ALK7-Fc	405	406
		B	ActRIIA-Fc	451	452
			ALK7-Fc	475	476
ActRIIB-Fc: ALK1-Fc	静电	A	ActRIIB-Fc	100	102
			ALK1-Fc	124	126
		B	ActRIIB-Fc	153	154
			ALK1-Fc	171	172
	疏水	A	ActRIIB-Fc	401	402
			ALK1-Fc	413	414
		B	ActRIIB-Fc	453	454
			ALK1-Fc	463	464
ActRIIB-Fc: ALK2-Fc	静电	A	ActRIIB-Fc	100	102
			ALK2-Fc	136	138
		B	ActRIIB-Fc	153	154
			ALK2-Fc	173	174
	疏水	A	ActRIIB-Fc	401	402
			ALK2-Fc	421	422
		B	ActRIIB-Fc	453	454
			ALK2-Fc	465	466
ActRIIB-Fc: ALK3-Fc	静电	A	ActRIIB-Fc	100	102
			ALK3-Fc	115	117
		B	ActRIIB-Fc	153	154
			ALK3-Fc	175	176
	疏水	A	ActRIIB-Fc	401	402
			ALK3-Fc	407	408
		B	ActRIIB-Fc	453	454
			ALK3-Fc	467	468
ActRIIB-Fc: ALK4-Fc	静电	A	ActRIIB-Fc	100	102
			ALK4-Fc	104	106
		B	ActRIIB-Fc	153	154
			ALK4-Fc	177	178
	疏水	A	ActRIIB-Fc	401	402
			ALK4-Fc	403	404
		B	ActRIIB-Fc	453	454
			ALK4-Fc	469	470

[1347]

ActRIIB-Fc: ALK5-Fc	静电	A	ActRIIB-Fc	100	102
			ALK5-Fc	139	141
		B	ActRIIB-Fc	153	154
			ALK5-Fc	179	180
	疏水	A	ActRIIB-Fc	401	402
			ALK5-Fc	423	424
		B	ActRIIB-Fc	453	454
			ALK5-Fc	471	472
ActRIIB-Fc: ALK6-Fc	静电	A	ActRIIB-Fc	100	102
			ALK6-Fc	142	144
		B	ActRIIB-Fc	153	154
			ALK6-Fc	181	182
	疏水	A	ActRIIB-Fc	401	402
			ALK6-Fc	425	426
		B	ActRIIB-Fc	453	454
			ALK6-Fc	473	474
ActRIIB-Fc: ALK7-Fc	静电	A	ActRIIB-Fc	100	102
			ALK7-Fc	112	114
		B	ActRIIB-Fc	153	154
			ALK7-Fc	183	184
	疏水	A	ActRIIB-Fc	401	402
			ALK7-Fc	405	406
		B	ActRIIB-Fc	453	454
			ALK7-Fc	475	476
BMPRII-Fc: ALK1-Fc	静电	A	BMPRII-Fc	121	123
			ALK1-Fc	124	126
		B	BMPRII-Fc	155	156
			ALK1-Fc	171	172
	疏水	A	BMPRII-Fc	411	412
			ALK1-Fc	413	414
		B	BMPRII-Fc	455	456
			ALK1-Fc	463	464
BMPRII-Fc: ALK2-Fc	静电	A	BMPRII-Fc	121	123
			ALK2-Fc	136	138
		B	BMPRII-Fc	155	156
			ALK2-Fc	173	174

[1348]

	疏水	A	BMPRII-Fc	411	412
			ALK2-Fc	421	422
		B	BMPRII-Fc	455	456
			ALK2-Fc	465	466
BMPRII-Fc: ALK3-Fc	静电	A	BMPRII-Fc	121	123
			ALK3-Fc	115	117
		B	BMPRII-Fc	155	156
			ALK3-Fc	175	176
	疏水	A	BMPRII-Fc	411	412
			ALK3-Fc	407	408
		B	BMPRII-Fc	455	456
			ALK3-Fc	467	468
BMPRII-Fc: ALK4-Fc	静电	A	BMPRII-Fc	121	123
			ALK4-Fc	104	106
		B	BMPRII-Fc	155	156
			ALK4-Fc	177	178
	疏水	A	BMPRII-Fc	411	412
			ALK4-Fc	403	404
		B	BMPRII-Fc	455	456
			ALK4-Fc	469	470
BMPRII-Fc: ALK5-Fc	静电	A	BMPRII-Fc	121	123
			ALK5-Fc	139	141
		B	BMPRII-Fc	155	156
			ALK5-Fc	179	180
	疏水	A	BMPRII-Fc	411	412
			ALK5-Fc	423	424
		B	BMPRII-Fc	455	456
			ALK5-Fc	471	472
BMPRII-Fc: ALK6-Fc	静电	A	BMPRII-Fc	121	123
			ALK6-Fc	142	144
		B	BMPRII-Fc	155	156
			ALK6-Fc	181	182
	疏水	A	BMPRII-Fc	411	412
			ALK6-Fc	425	426
		B	BMPRII-Fc	455	456
			ALK6-Fc	473	474

[1349]

BMPRII-Fc: ALK7-Fc	静电	A	BMPRII-Fc	121	123
			ALK7-Fc	112	114
		B	BMPRII-Fc	155	156
			ALK7-Fc	183	184
	疏水	A	BMPRII-Fc	411	412
			ALK7-Fc	405	406
		B	BMPRII-Fc	455	456
			ALK7-Fc	475	476
MISRII-Fc: ALK1-Fc	静电	A	MISRII-Fc	133	135
			ALK1-Fc	124	126
		B	MISRII-Fc	161	162
			ALK1-Fc	171	172
	疏水	A	MISRII-Fc	419	420
			ALK1-Fc	413	414
		B	MISRII-Fc	457	458
			ALK1-Fc	463	464
MISRII-Fc: ALK2-Fc	静电	A	MISRII-Fc	133	135
			ALK2-Fc	136	138
		B	MISRII-Fc	161	162
			ALK2-Fc	173	174
	疏水	A	MISRII-Fc	419	420
			ALK2-Fc	421	422
		B	MISRII-Fc	457	458
			ALK2-Fc	465	466
MISRII-Fc: ALK3-Fc	静电	A	MISRII-Fc	133	135
			ALK3-Fc	115	117
		B	MISRII-Fc	161	162
			ALK3-Fc	175	176
	疏水	A	MISRII-Fc	419	420
			ALK3-Fc	407	408
		B	MISRII-Fc	457	458
			ALK3-Fc	467	468
MISRII-Fc: ALK4-Fc	静电	A	MISRII-Fc	133	135
			ALK4-Fc	104	106
		B	MISRII-Fc	161	162
			ALK4-Fc	177	178

[1350]

	疏水	A	MISRII-Fc	419	420
			ALK4-Fc	403	404
		B	MISRII-Fc	457	458
			ALK4-Fc	469	470
MISRII-Fc: ALK5-Fc	静电	A	MISRII-Fc	133	135
			ALK5-Fc	139	141
		B	MISRII-Fc	161	162
			ALK5-Fc	179	180
	疏水	A	MISRII-Fc	419	420
			ALK5-Fc	423	424
		B	MISRII-Fc	457	458
			ALK5-Fc	471	472
MISRII-Fc: ALK6-Fc	静电	A	MISRII-Fc	133	135
			ALK6-Fc	142	144
		B	MISRII-Fc	161	162
			ALK6-Fc	181	182
	疏水	A	MISRII-Fc	419	420
			ALK6-Fc	425	426
		B	MISRII-Fc	457	458
			ALK6-Fc	473	474
MISRII-Fc: ALK7-Fc	静电	A	MISRII-Fc	133	135
			ALK7-Fc	112	114
		B	MISRII-Fc	161	162
			ALK7-Fc	183	184
	疏水	A	MISRII-Fc	419	420
			ALK7-Fc	405	406
		B	MISRII-Fc	457	458
			ALK7-Fc	475	476
$TGF\beta RII_{SHORT}$-Fc: ALK1-Fc	静电	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK1-Fc	124	126
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK1-Fc	171	172
	疏水	A	$TGF\beta RII_{SHORT}$-Fc	415	416

[1351]

			ALK1-Fc	413	414
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK1-Fc	463	464
$TGF\beta RII_{SHORT}$-Fc: ALK2-Fc	静电	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK2-Fc	136	138
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK2-Fc	173	174
	疏水	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK2-Fc	421	422
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK2-Fc	465	466
$TGF\beta RII_{SHORT}$-Fc: ALK3-Fc	静电	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK3-Fc	115	117
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK3-Fc	175	176
	疏水	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK3-Fc	407	408
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK3-Fc	467	468
$TGF\beta RII_{SHORT}$-Fc: ALK4-Fc	静电	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK4-Fc	104	106
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK4-Fc	177	178
	疏水	A	$TGF\beta RII_{SHORT}$-Fc	415	416

[1352]

			ALK4-Fc	403	404
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK4-Fc	469	470
$TGF\beta RII_{SHORT}$-Fc: ALK5-Fc	静电	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK5-Fc	139	141
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK5-Fc	179	180
	疏水	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK5-Fc	423	424
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK5-Fc	471	472
$TGF\beta RII_{SHORT}$-Fc: ALK6-Fc	静电	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK6-Fc	142	144
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK6-Fc	181	182
	疏水	A	$TGF\beta RII_{SHORT}$-Fc	415	416
			ALK6-Fc	425	426
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK6-Fc	473	474
$TGF\beta RII_{SHORT}$-Fc: ALK7-Fc	静电	A	$TGF\beta RII_{SHORT}$-Fc	127	129
			ALK7-Fc	112	114
		B	$TGF\beta RII_{SHORT}$-Fc	157	158
			ALK7-Fc	183	184
	疏水	A	$TGF\beta RII_{SHORT}$-Fc	415	416

[1353]

			ALK7-Fc	405	406
		B	$TGF\beta RII_{SHORT}$-Fc	459	460
			ALK7-Fc	475	476
$TGF\beta RII_{LONG}$-Fc : ALK1-Fc	静电	A	$TGF\beta RII_{LONG}$-Fc	130	132
			ALK1-Fc	124	126
		B	$TGF\beta RII_{LONG}$-Fc	159	160
			ALK1-Fc	171	172
	疏水	A	$TGF\beta RII_{LONG}$-Fc	417	418
			ALK1-Fc	413	414
		B	$TGF\beta RII_{LONG}$-Fc	461	462
			ALK1-Fc	463	464
$TGF\beta RII_{LONG}$-Fc : ALK2-Fc	静电	A	$TGF\beta RII_{LONG}$-Fc	130	132
			ALK2-Fc	136	138
		B	$TGF\beta RII_{LONG}$-Fc	159	160
			ALK2-Fc	173	174
	疏水	A	$TGF\beta RII_{LONG}$-Fc	417	418
			ALK2-Fc	421	422
		B	$TGF\beta RII_{LONG}$-Fc	461	462
			ALK2-Fc	465	466
$TGF\beta RII_{LONG}$-Fc : ALK3-Fc	静电	A	$TGF\beta RII_{LONG}$-Fc	130	132
			ALK3-Fc	115	117
		B	$TGF\beta RII_{LONG}$-Fc	159	160
			ALK3-Fc	175	176
	疏水	A	$TGF\beta RII_{LONG}$-Fc	417	418
			ALK3-Fc	407	408
		B	$TGF\beta RII_{LONG}$-Fc	461	462
			ALK3-Fc	467	468
$TGF\beta RII_{LONG}$-Fc : ALK4-Fc	静电	A	$TGF\beta RII_{LONG}$-Fc	130	132
			ALK4-Fc	104	106
		B	$TGF\beta RII_{LONG}$-Fc	159	160
			ALK4-Fc	177	178
	疏水	A	$TGF\beta RII_{LONG}$-Fc	417	418
			ALK4-Fc	403	404
		B	$TGF\beta RII_{LONG}$-Fc	461	462
			ALK4-Fc	469	470

[1354]

TGFβRII$_{LONG}$-Fc : ALK5-Fc	静电	A	TGFβRII$_{LONG}$-Fc	130	132
			ALK5-Fc	139	141
		B	TGFβRII$_{LONG}$-Fc	159	160
			ALK5-Fc	179	180
	疏水	A	TGFβRII$_{LONG}$-Fc	417	418
			ALK5-Fc	423	424
		B	TGFβRII$_{LONG}$-Fc	461	462
			ALK5-Fc	471	472
TGFβRII$_{LONG}$-Fc : ALK6-Fc	静电	A	TGFβRII$_{LONG}$-Fc	130	132
			ALK6-Fc	142	144
		B	TGFβRII$_{LONG}$-Fc	159	160
			ALK6-Fc	181	182
	疏水	A	TGFβRII$_{LONG}$-Fc	417	418
			ALK6-Fc	425	426
		B	TGFβRII$_{LONG}$-Fc	461	462
			ALK6-Fc	473	474
TGFβRII$_{LONG}$-Fc : ALK7-Fc	静电	A	TGFβRII$_{LONG}$-Fc	130	132
			ALK7-Fc	112	114
		B	TGFβRII$_{LONG}$-Fc	159	160
			ALK7-Fc	183	184
	疏水	A	TGFβRII$_{LONG}$-Fc	417	418
			ALK7-Fc	405	406
		B	TGFβRII$_{LONG}$-Fc	461	462
			ALK7-Fc	475	476

[1355] 总体来说,这些实施例表明当置于异聚体复合体的情况下,I型和II型受体多肽形成新的结合袋,其显示相对于任一类型的同聚体复合体选择性改变,使得形成可能用作治疗剂的新的蛋白质作用剂。

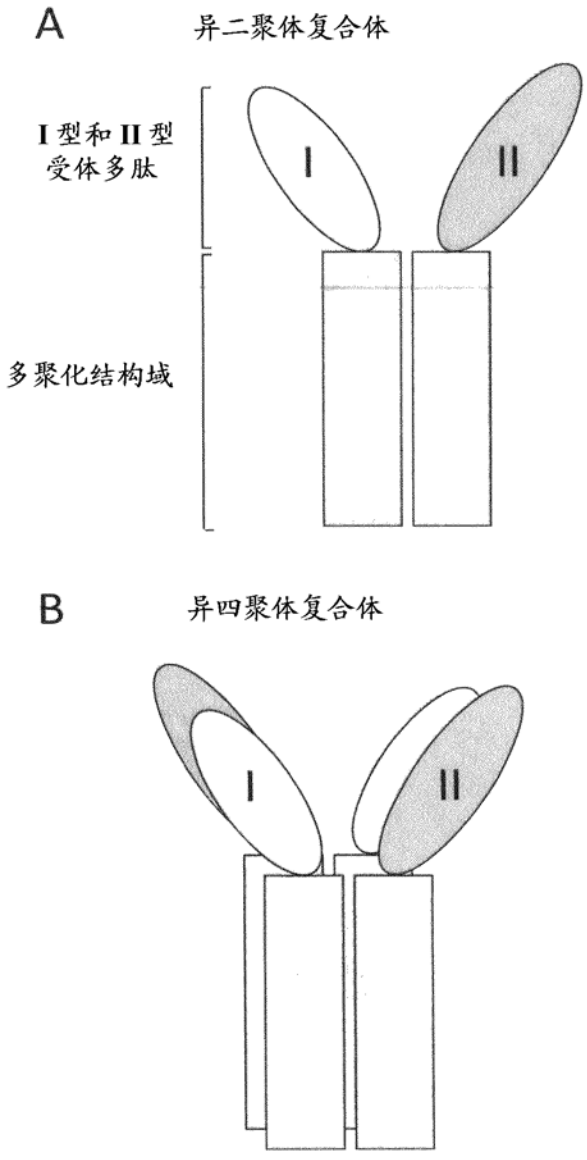


图 1

异聚体蛋白质复合体示意图

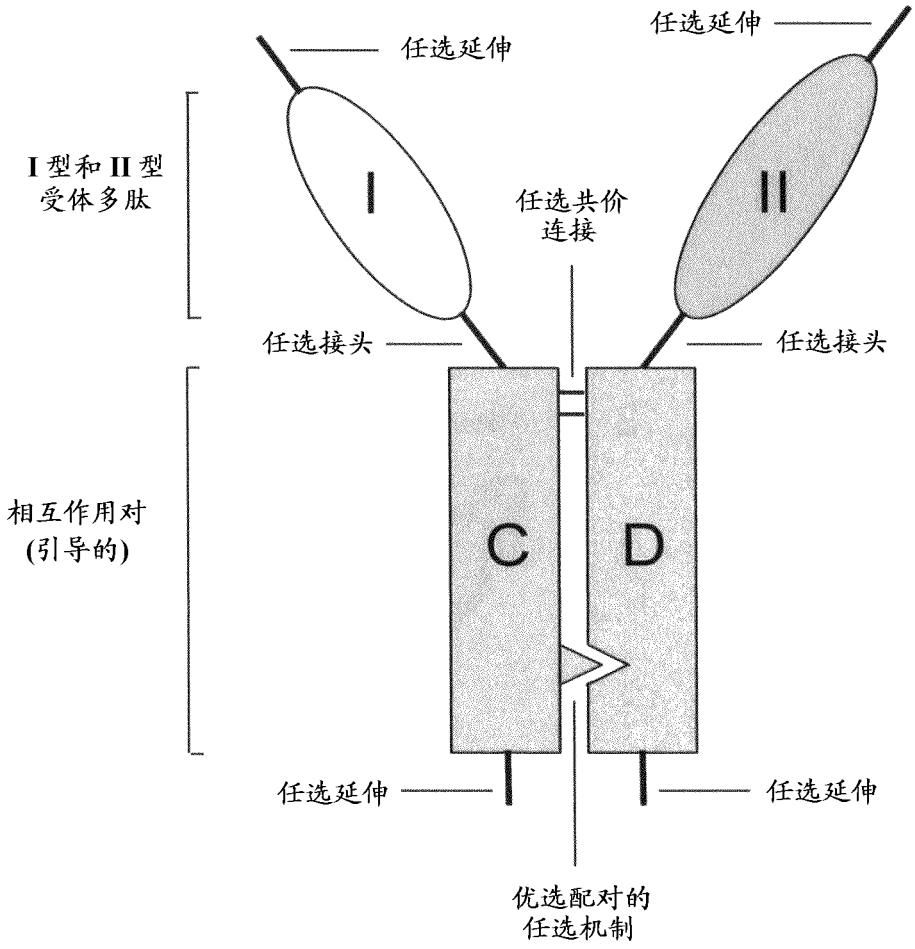


图 2

```
ActRIIa         ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
ActRIIb         GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT

                IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
                IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA

                EVTQPTSNPV TPKPPT
                GGPEVTYEPP PTAPT
```

图 3

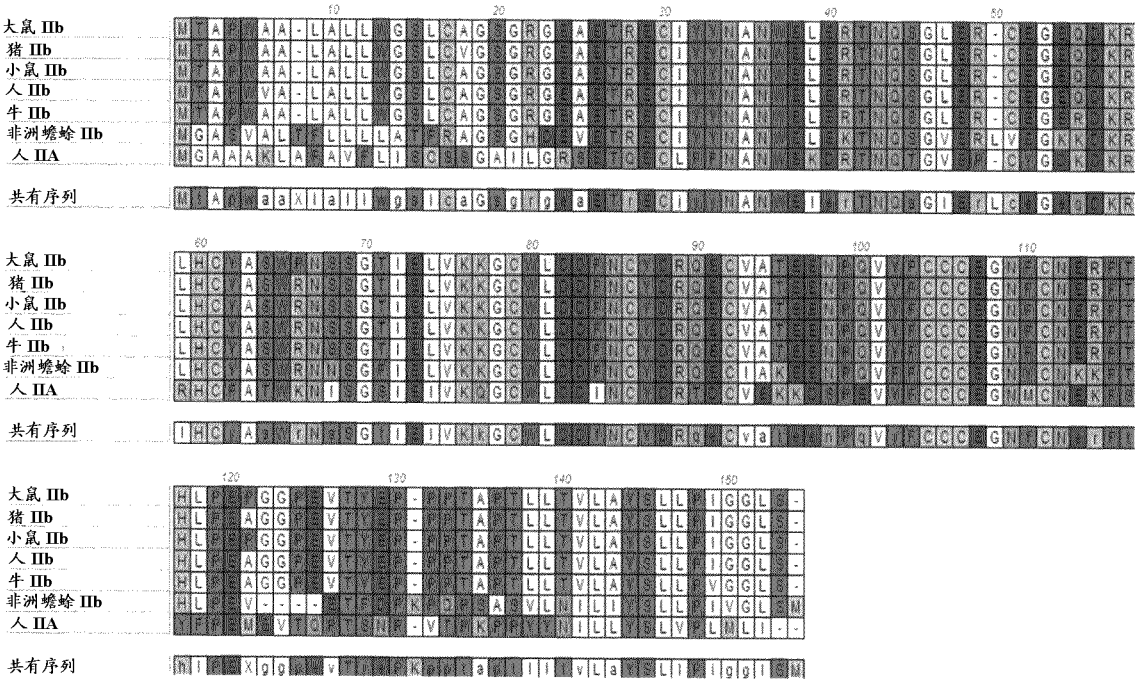


图 4

```
IgG1   -------THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF 53
IgG4   ---ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF 57
IgG2   --------VECPPCPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 51
IgG3   EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 60
                  ** **** . * ********************************:*****:*

IgG1   NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 113
IgG4   NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT 117
IgG2   NWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT 111
IgG3   KWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 120
       :********************:***:********:*****************.**:.****

IgG1   ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 173
IgG4   ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 177
IgG2   ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 171
IgG3   ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTP 180
       ***:***************:****************************.*******:***

IgG1   PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 225
IgG4   PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK 229
IgG2   PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 223
IgG3   PMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK 232
       *:***********:*********:**:************::******** **
```

图 5

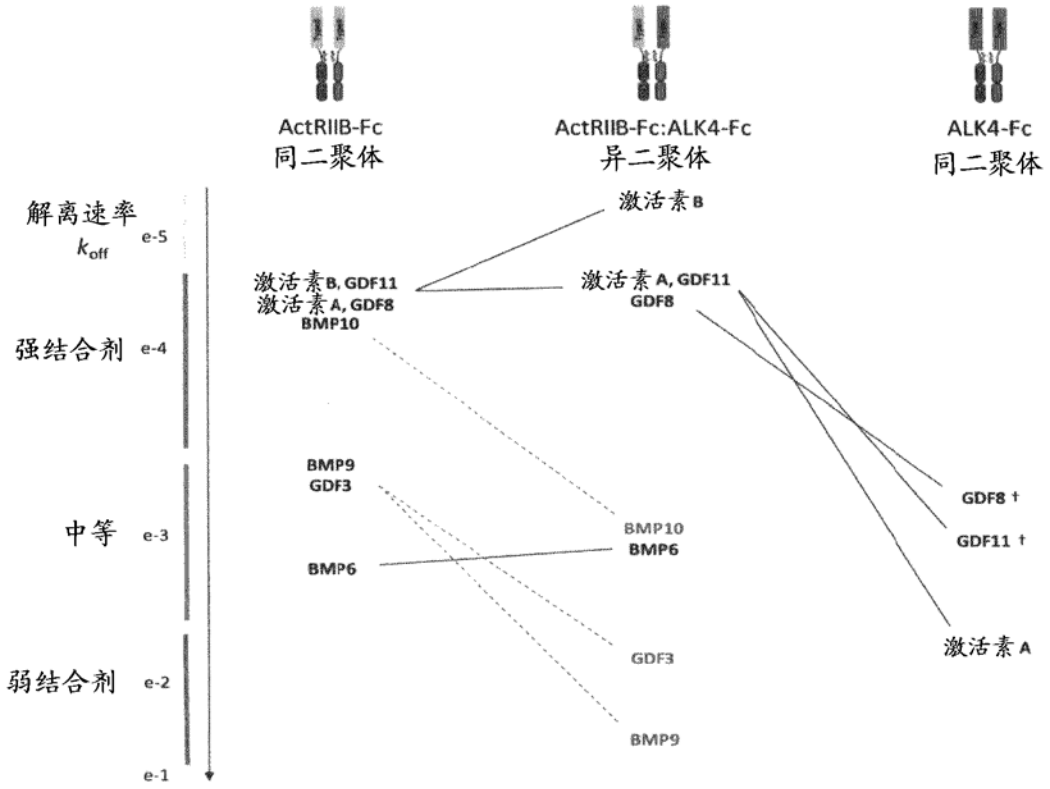

ActRIIB-Fc
同二聚体
ActRIIB-Fc:ALK4-Fc
异二聚体
ALK4-Fc
同二聚体
解离速率
k_{off}
e-5
强结合剂
e-4
中等
e-3
弱结合剂
e-2
e-1
激活素B
激活素B, GDF11
激活素A, GDF8
BMP10
激活素A, GDF11
GDF8
BMP9
GDF3
BMP10
BMP6
BMP6
GDF3
BMP9
GDF8 †
GDF11 †
激活素A

图 6

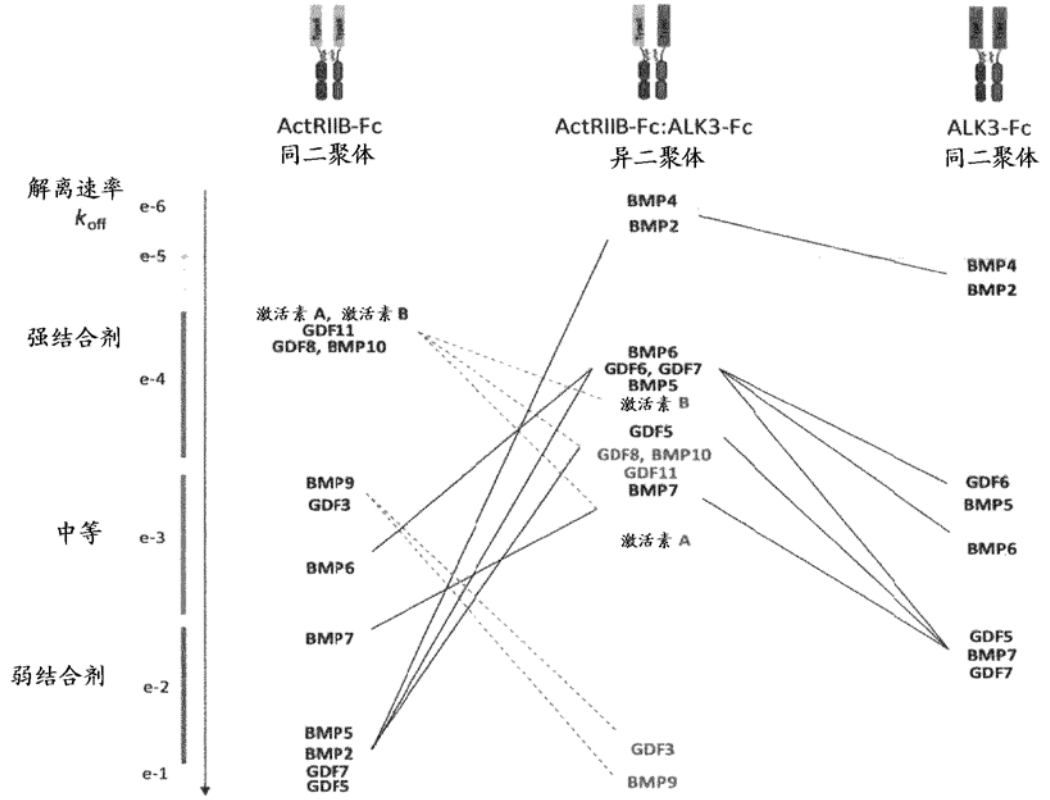

ActRIIB-Fc
同二聚体
ActRIIB-Fc:ALK3-Fc
异二聚体
ALK3-Fc
同二聚体
解离速率
k_{off}
e-6
e-5
强结合剂
e-4
中等
e-3
弱结合剂
e-2
e-1
BMP4
BMP2
BMP4
BMP2
激活素A, 激活素B
GDF11
GDF8, BMP10
BMP6
GDF6, GDF7
BMP5
激活素B
GDF5
GDF8, BMP10
GDF11
BMP7
激活素A
BMP9
GDF3
BMP6
BMP7
BMP5
BMP2
GDF7
GDF5
GDF6
BMP5
BMP6
GDF5
BMP7
GDF7
GDF3
BMP9

图 7

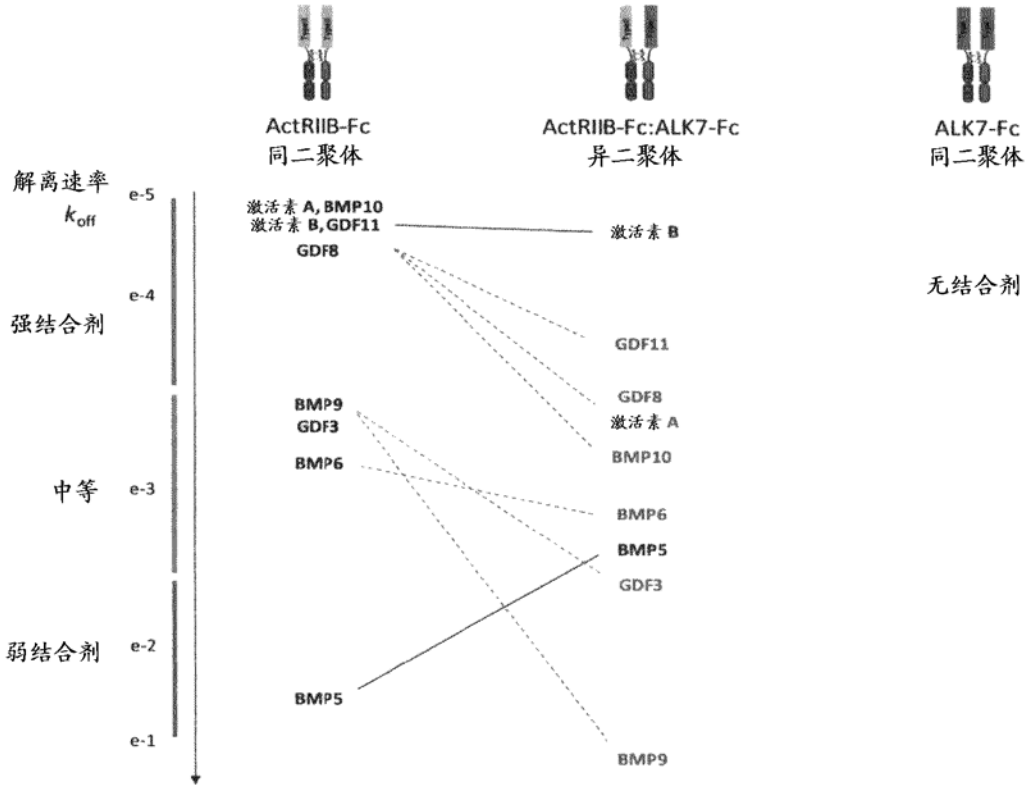

ActRIIB-Fc
同二聚体
ActRIIB-Fc:ALK7-Fc
异二聚体
ALK7-Fc
同二聚体
解离速率
k_{off}
e-5
e-4
e-3
e-2
e-1
强结合剂
中等
弱结合剂
激活素 A, BMP10
激活素 B, GDF11
GDF8
BMP9
GDF3
BMP6
BMP5
激活素 B
GDF11
GDF8
激活素 A
BMP10
BMP6
BMP5
GDF3
BMP9
无结合剂

图 8

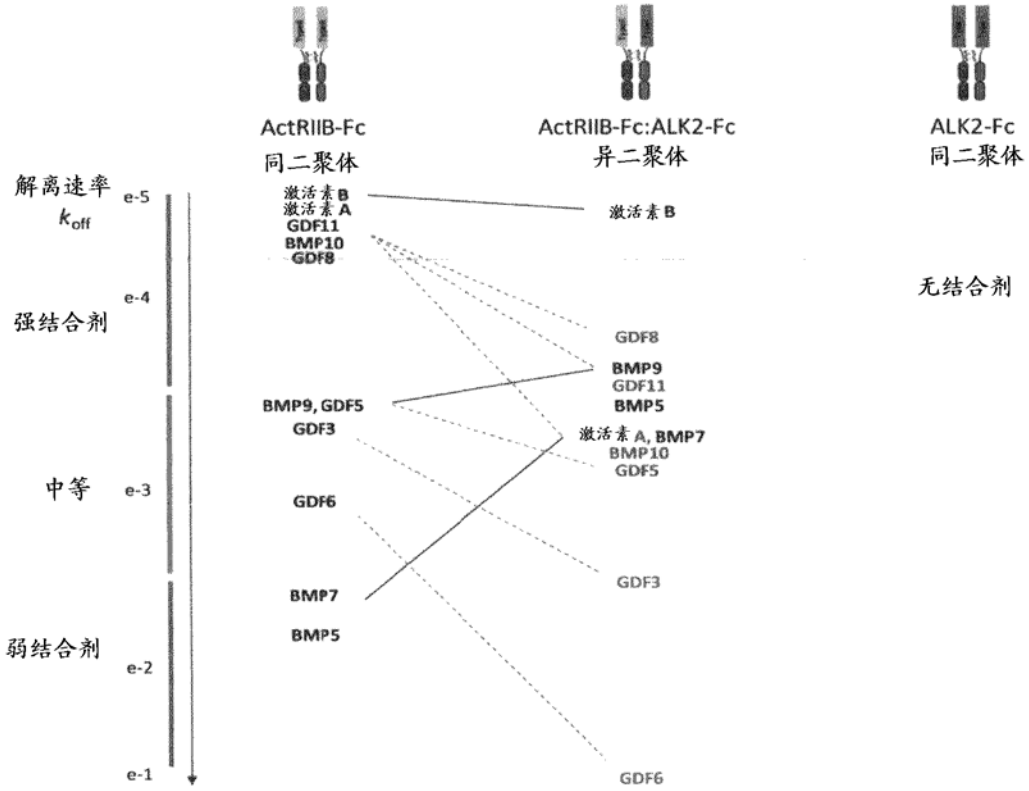

ActRIIB-Fc
同二聚体
ActRIIB-Fc:ALK2-Fc
异二聚体
ALK2-Fc
同二聚体
解离速率
k_{off}
e-5
e-4
e-3
e-2
e-1
强结合剂
中等
弱结合剂
激活素B
激活素A
GDF11
BMP10
GDF8
BMP9, GDF5
GDF3
GDF6
BMP7
BMP5
激活素 B
GDF8
BMP9
GDF11
BMP5
激活素 A, BMP7
BMP10
GDF5
GDF3
GDF6
无结合剂

图 9

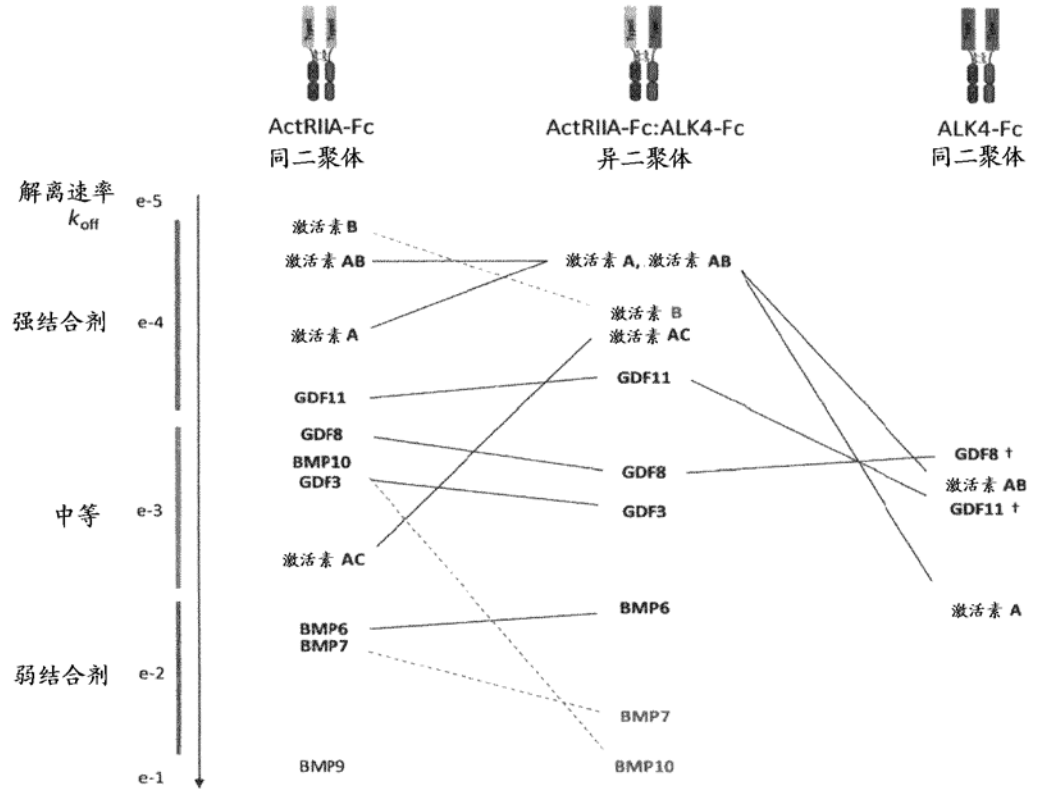

ActRIIA-Fc
同二聚体
ActRIIA-Fc:ALK4-Fc
异二聚体
ALK4-Fc
同二聚体
解离速率
k_{off}
e-5
强结合剂
e-4
中等
e-3
弱结合剂
e-2
e-1
激活素B
激活素AB
激活素A
激活素AC
GDF11
GDF8
BMP10
GDF3
BMP6
BMP7
BMP9
激活素A, 激活素AB
激活素B
激活素AC
GDF11
GDF8
GDF3
BMP6
BMP7
BMP10
GDF8 †
激活素AB
GDF11 †
激活素A

图 10

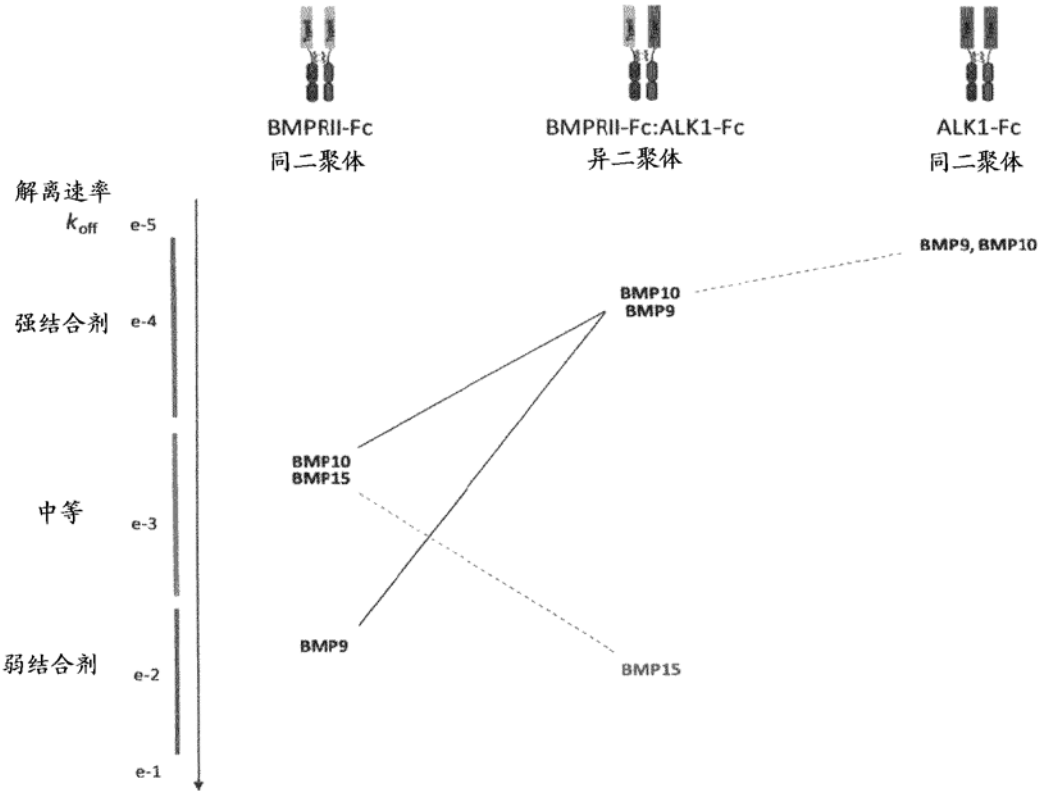

BMPRII-Fc
同二聚体
BMPRII-Fc:ALK1-Fc
异二聚体
ALK1-Fc
同二聚体
解离速率
k_{off}
e-5
强结合剂
e-4
中等
e-3
弱结合剂
e-2
e-1
BMP10
BMP15
BMP9
BMP9, BMP10
BMP10
BMP9
BMP15

图 11

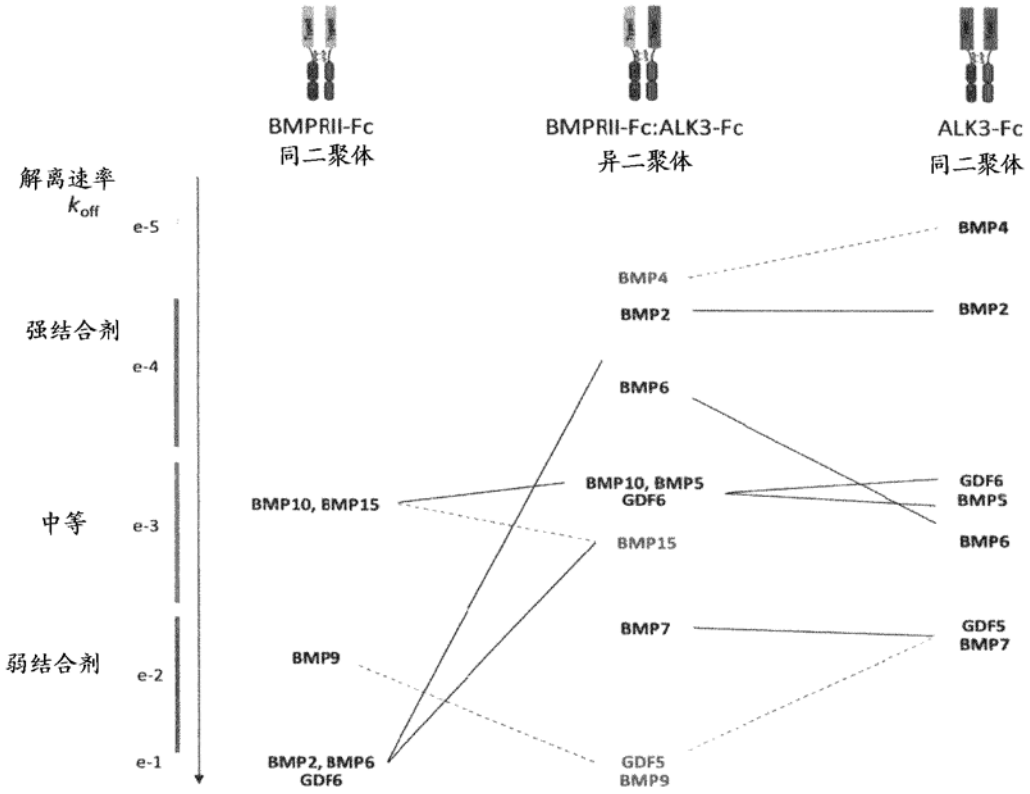

BMPRII-Fc
同二聚体
BMPRII-Fc:ALK3-Fc
异二聚体
ALK3-Fc
同二聚体
解离速率
k_{off}
e-5
强结合剂
e-4
中等
e-3
弱结合剂
e-2
e-1
BMP4
BMP2
BMP6
BMP10, BMP5
GDF6
BMP15
BMP7
GDF5
BMP7
BMP9
GDF5
BMP9
BMP10, BMP15
BMP2, BMP6
GDF6
BMP4
BMP2
GDF6
BMP5
BMP6
GDF5
BMP7

图 12

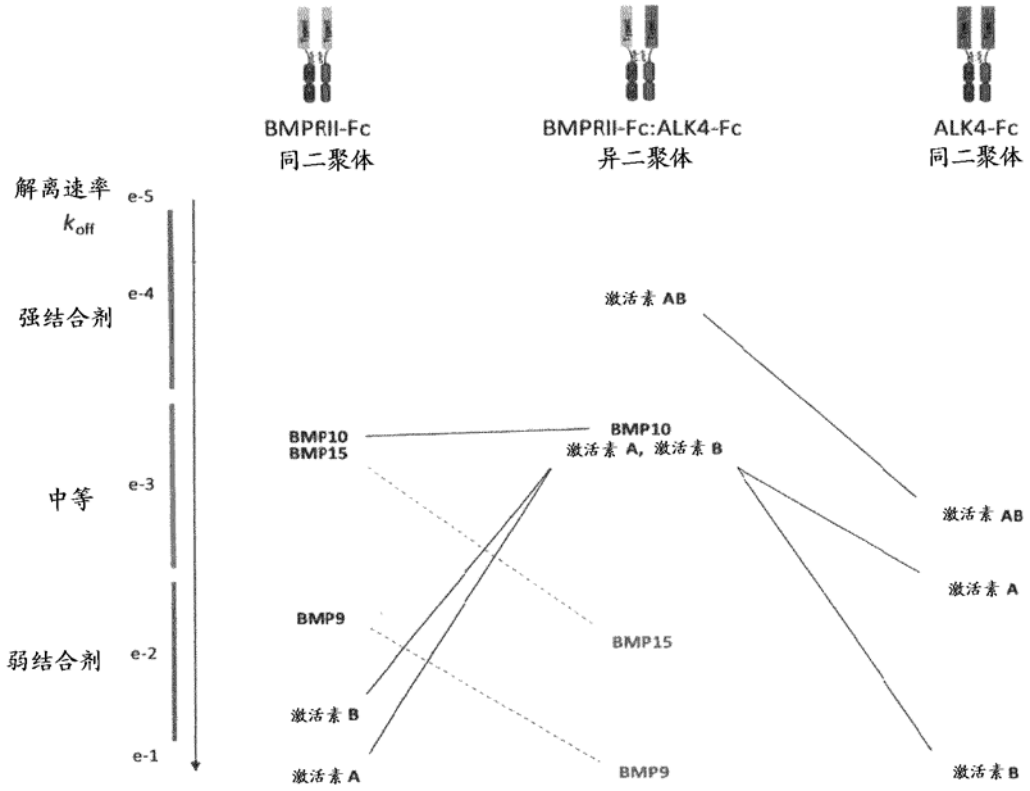

BMPRII-Fc
同二聚体
BMPRII-Fc:ALK4-Fc
异二聚体
ALK4-Fc
同二聚体
解离速率
k_{off}
e-5
强结合剂
e-4
中等
e-3
弱结合剂
e-2
e-1
激活素 AB
BMP10
激活素 A, 激活素 B
BMP15
BMP9
BMP10
BMP15
BMP9
激活素 B
激活素 A
激活素 AB
激活素 A
激活素 B

图 13

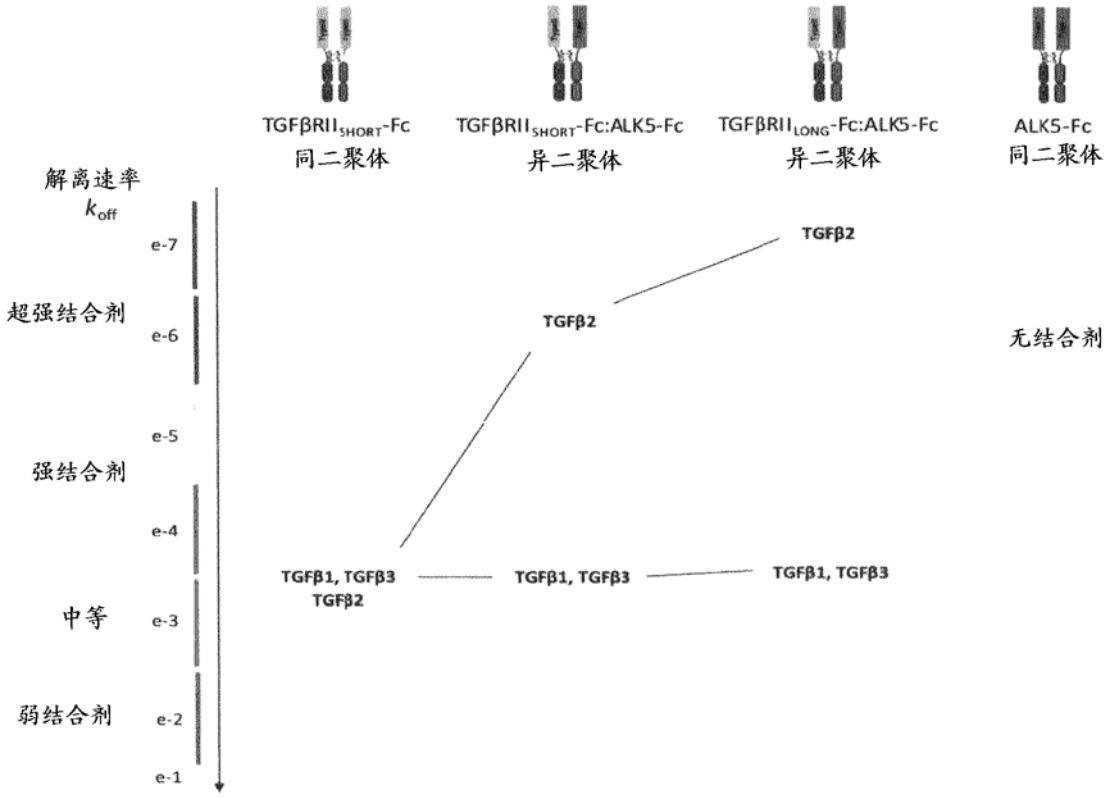

TGFβRII$_{SHORT}$-Fc
同二聚体
TGFβRII$_{SHORT}$-Fc:ALK5-Fc
异二聚体
TGFβRII$_{LONG}$-Fc:ALK5-Fc
异二聚体
ALK5-Fc
同二聚体
解离速率
k_{off}
e-7
超强结合剂
e-6
e-5
强结合剂
e-4
e-3
中等
弱结合剂
e-2
e-1
TGFβ2
TGFβ2
无结合剂
TGFβ1, TGFβ3
TGFβ2
TGFβ1, TGFβ3
TGFβ1, TGFβ3

图 14

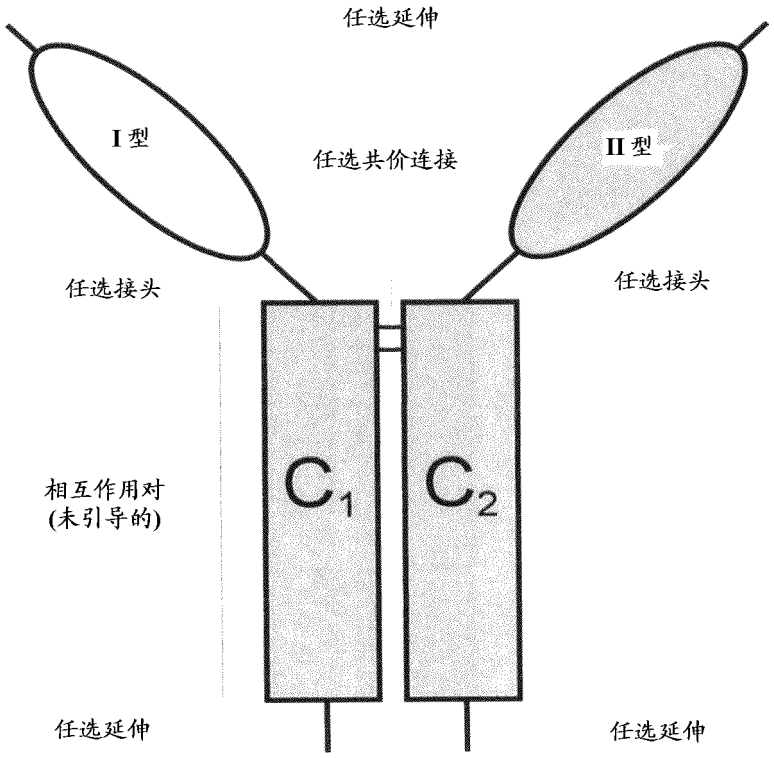

任选延伸
I 型
任选共价连接
II 型
任选接头
任选接头
相互作用对
(未引导的)
C_1
C_2
任选延伸
任选延伸

图 15A

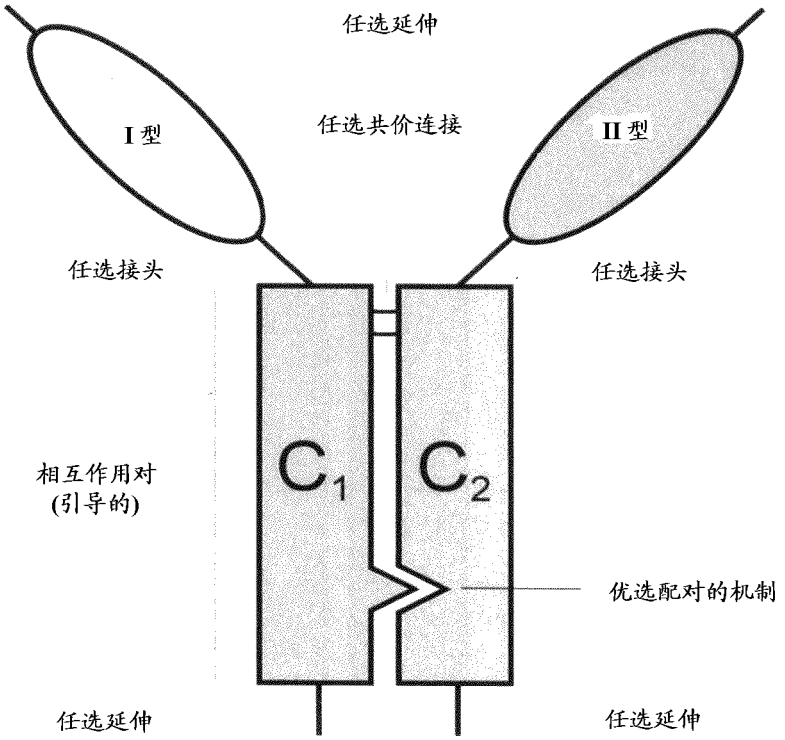

任选延伸
I 型
任选共价连接
II 型
任选接头
任选接头
相互作用对
(引导的)
C1
C2
优选配对的机制
任选延伸
任选延伸

图15B

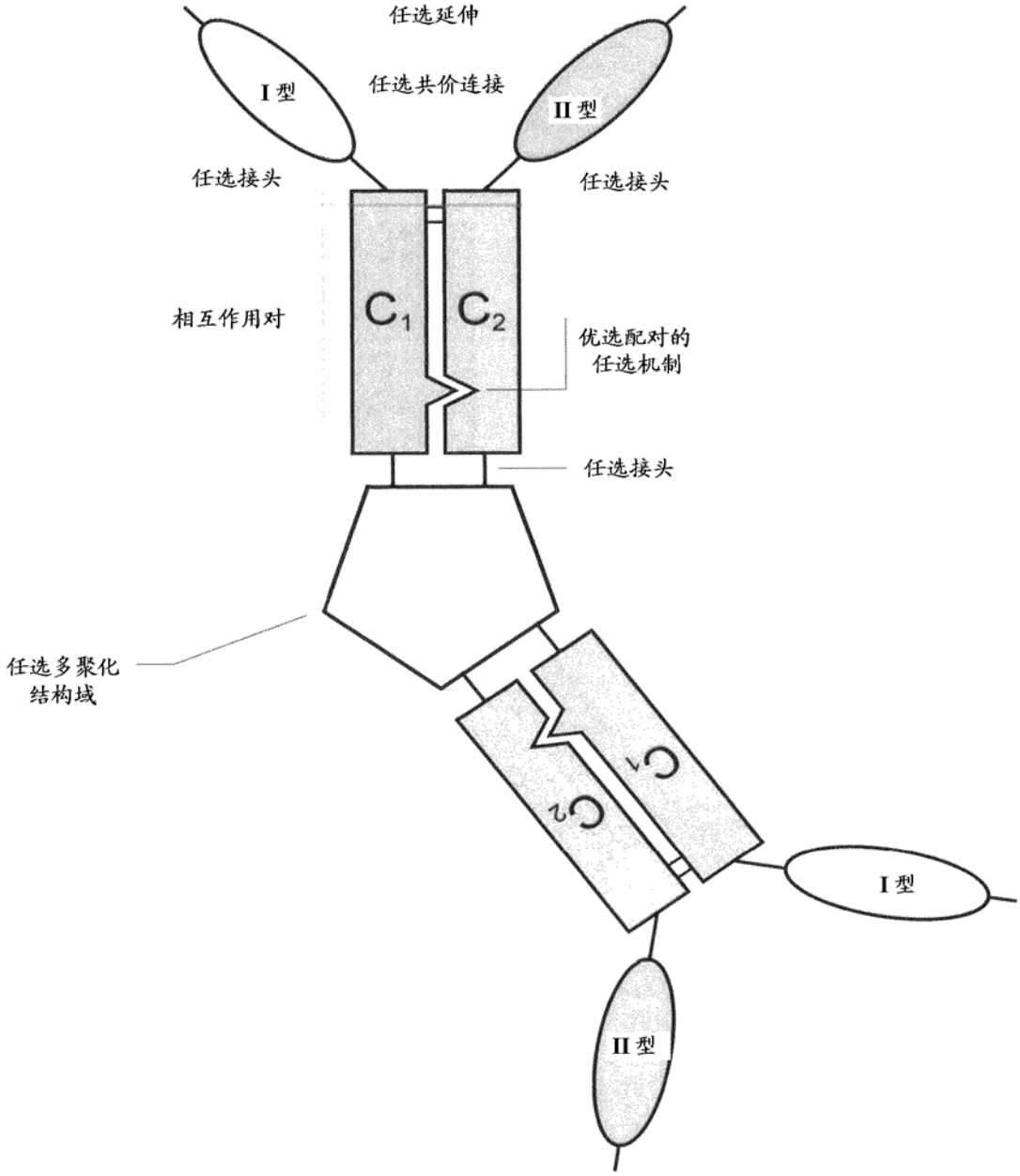

任选延伸
I型
任选共价连接
II型
任选接头
任选接头
相互作用对
C1
C2
优选配对的
任选机制
任选接头
任选多聚化
结构域
C1
C2
I型
II型

图 15C

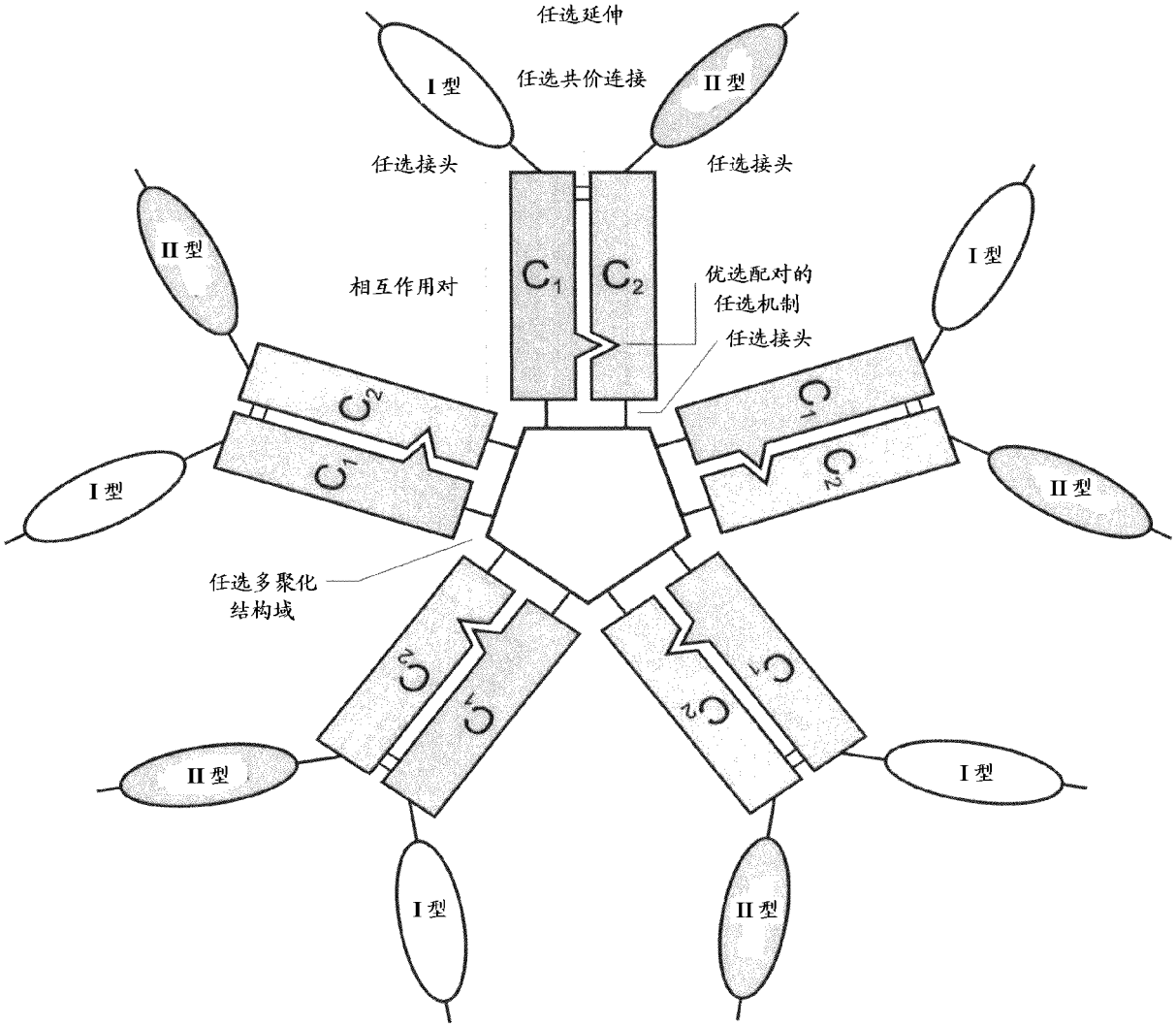

任选延伸
I 型
任选共价连接
II 型
任选接头
任选接头
II 型
相互作用对
C1
C2
优选配对的
任选机制
任选接头
I 型
C2
C1
C1
C2
I 型
II 型
任选多聚化
结构域
C2
C1
C1
C2
II 型
I 型
I 型
II 型

图 15D

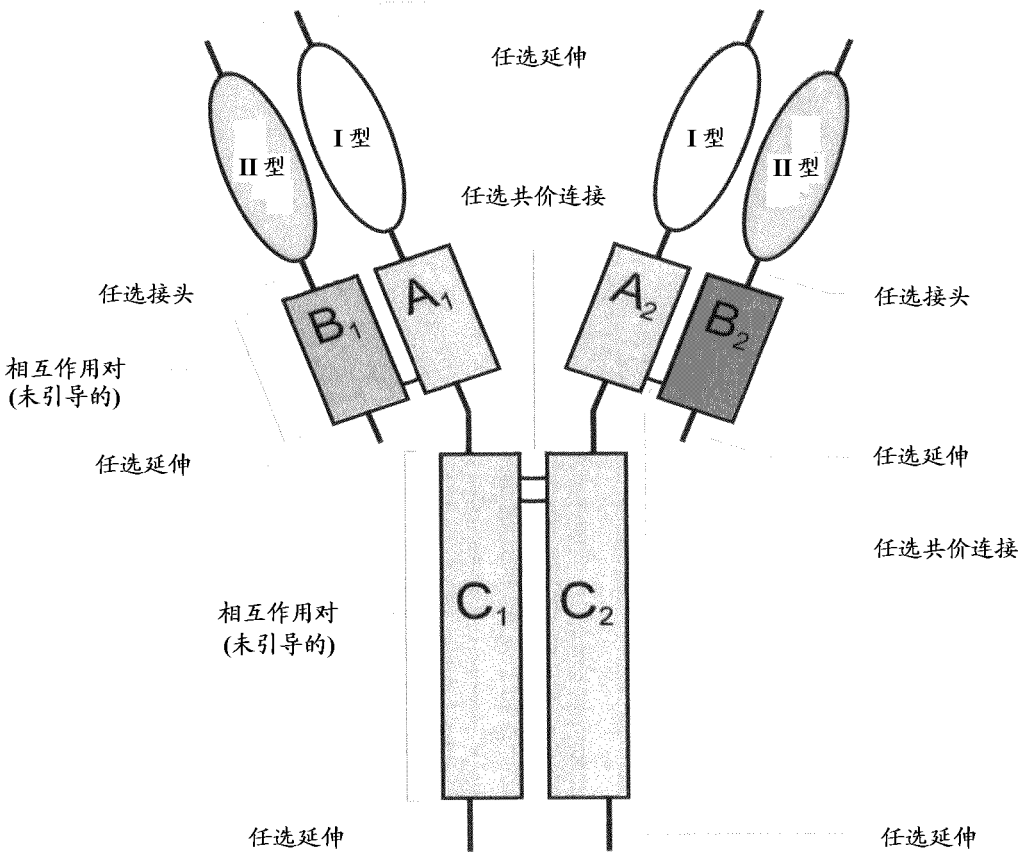

任选延伸
I 型
II 型
任选共价连接
I 型
II 型
任选接头
任选接头
相互作用对
(未引导的)
任选延伸
任选延伸
任选共价连接
相互作用对
(未引导的)
任选延伸
任选延伸

图 16A

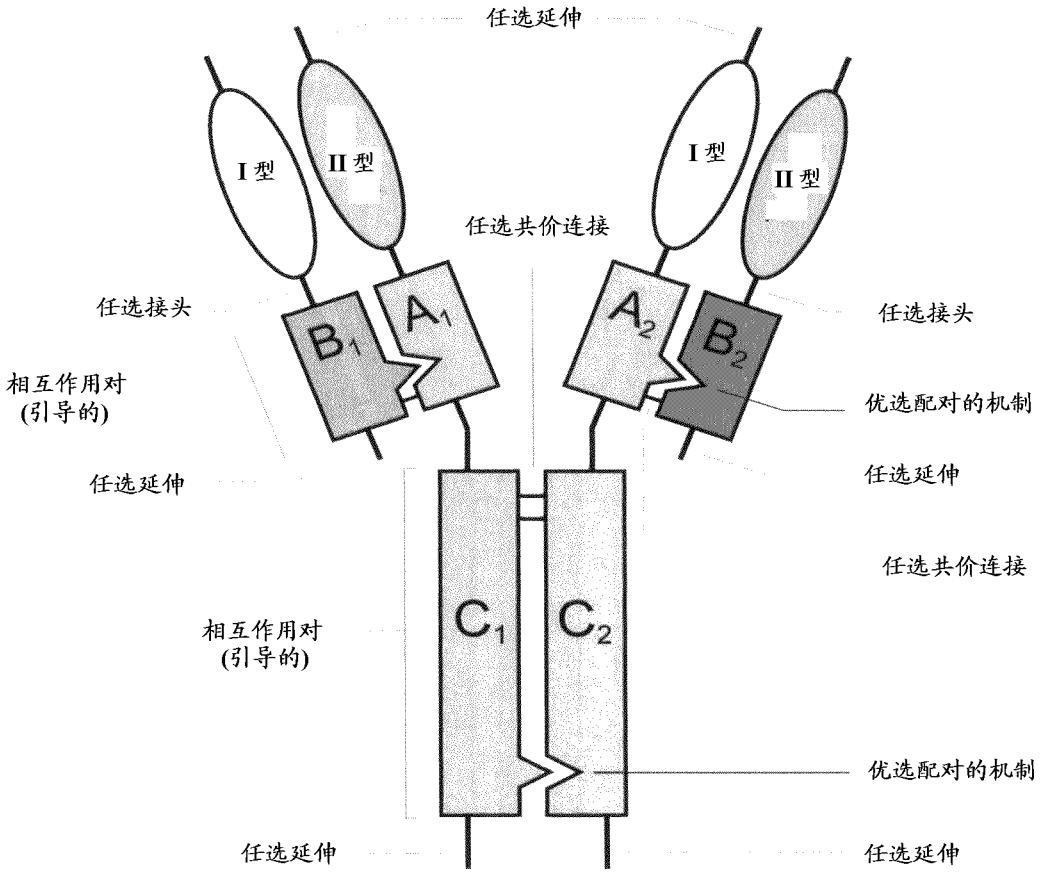

任选延伸
I 型
II 型
I 型
II 型
任选共价连接
任选接头
任选接头
相互作用对
(引导的)
优选配对的机制
任选延伸
任选延伸
任选共价连接
相互作用对
(引导的)
优选配对的机制
任选延伸
任选延伸

图 16B

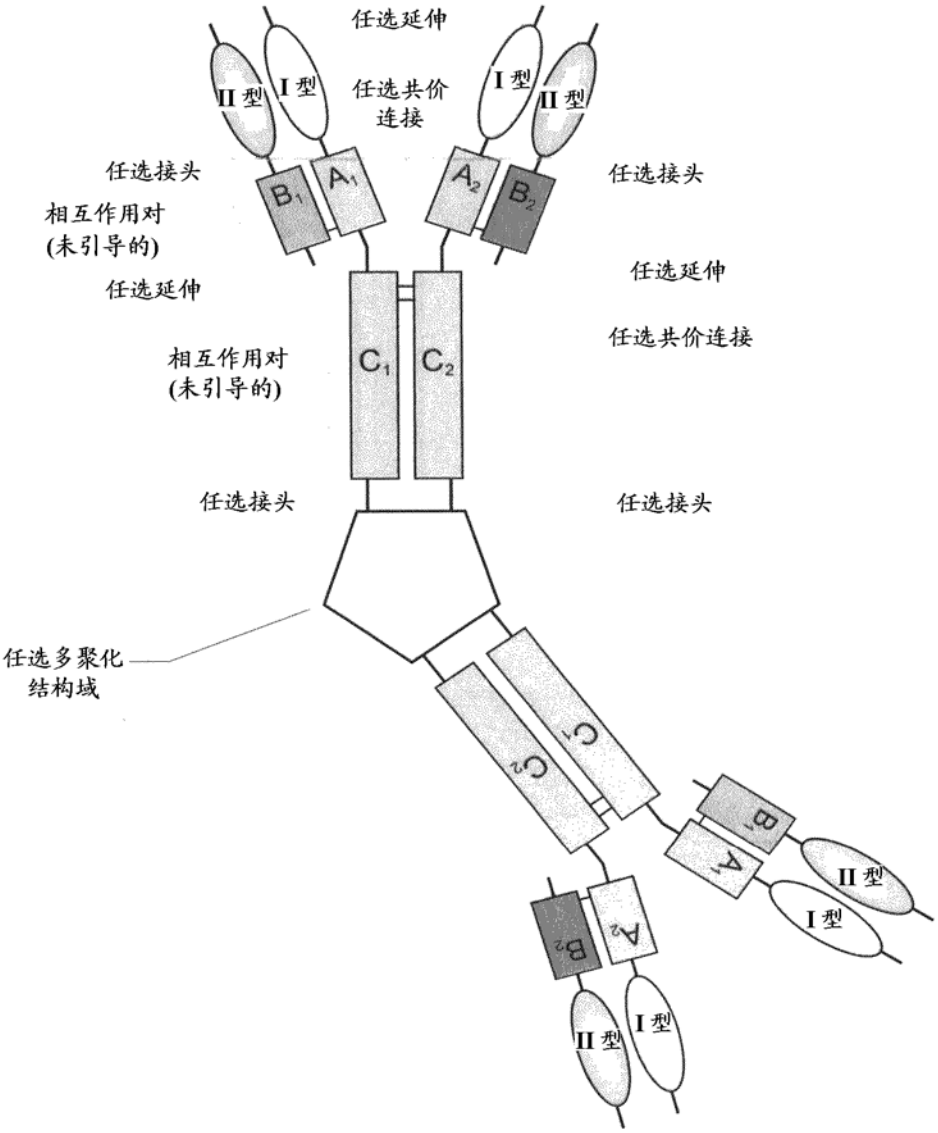

任选延伸
II 型
I 型
任选共价
连接
I 型
II 型
任选接头
B1
A1
A2
B2
任选接头
相互作用对
(未引导的)
任选延伸
任选延伸
任选共价连接
相互作用对
(未引导的)
C1
C2
任选接头
任选接头
任选多聚化
结构域
C1
C2
B1
A1
II 型
I 型
B2
A2
II 型
I 型

图 16C

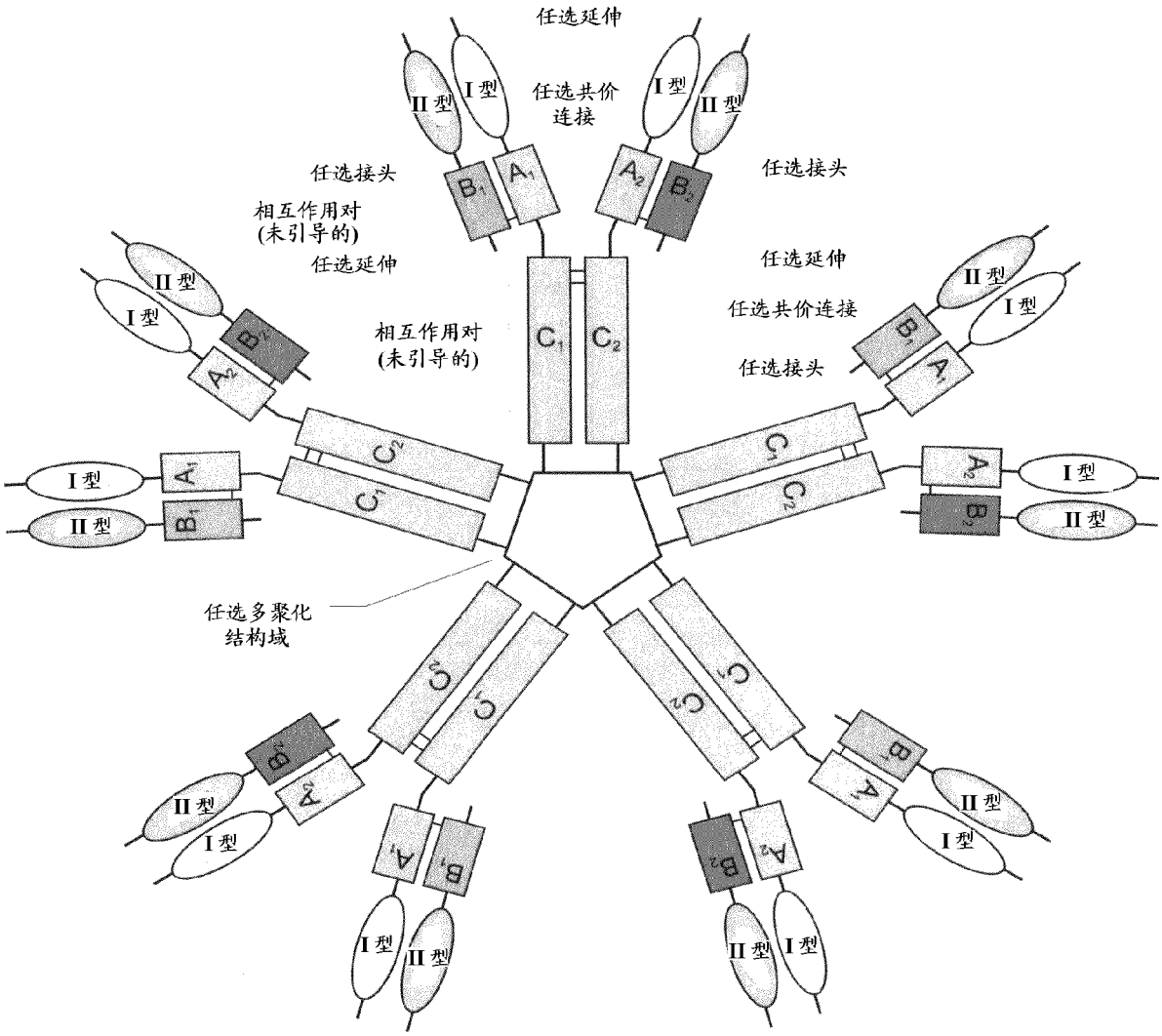


图 16D

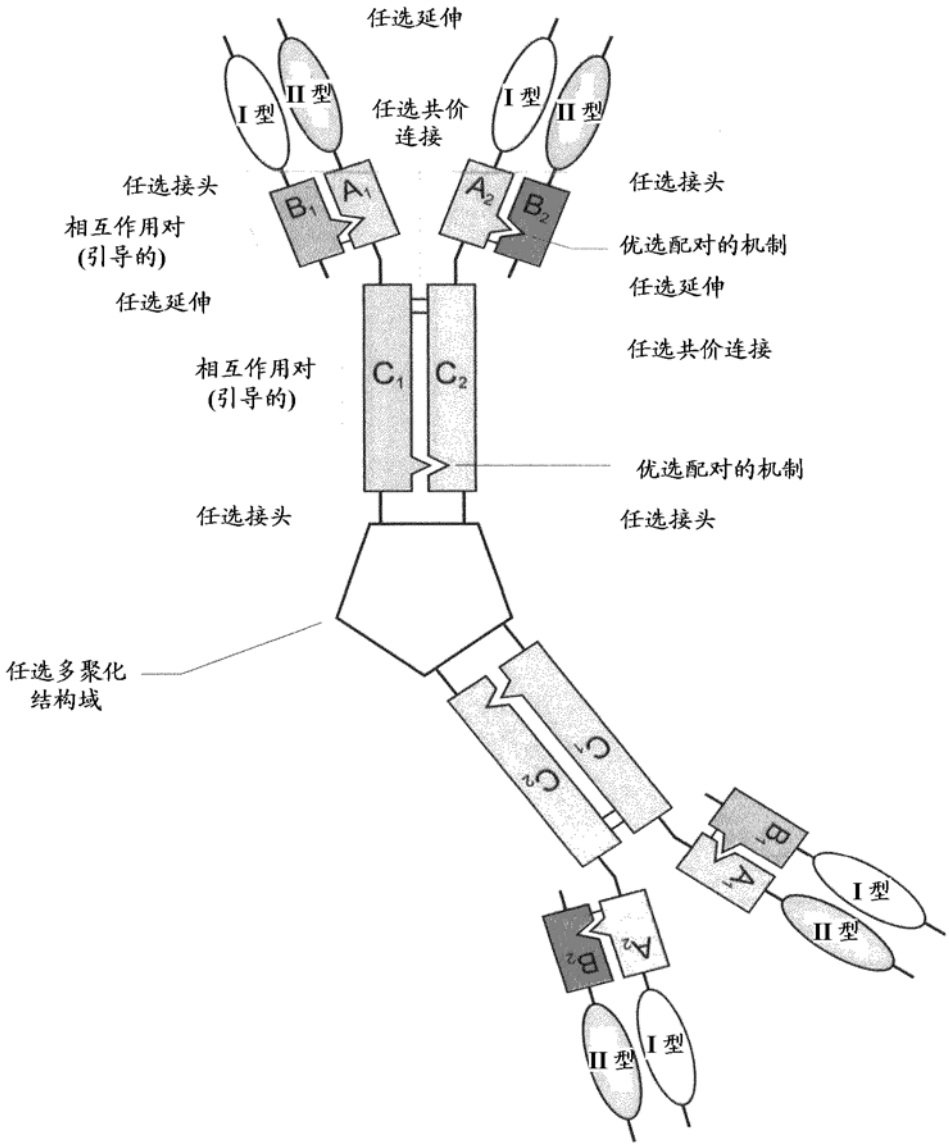

任选延伸
I 型
II 型
任选共价连接
I 型
II 型
任选接头
任选接头
相互作用对(引导的)
优选配对的机制
任选延伸
任选延伸
任选共价连接
相互作用对(引导的)
优选配对的机制
任选接头
任选接头
任选多聚化结构域
I 型
II 型
II 型
I 型

图 16E

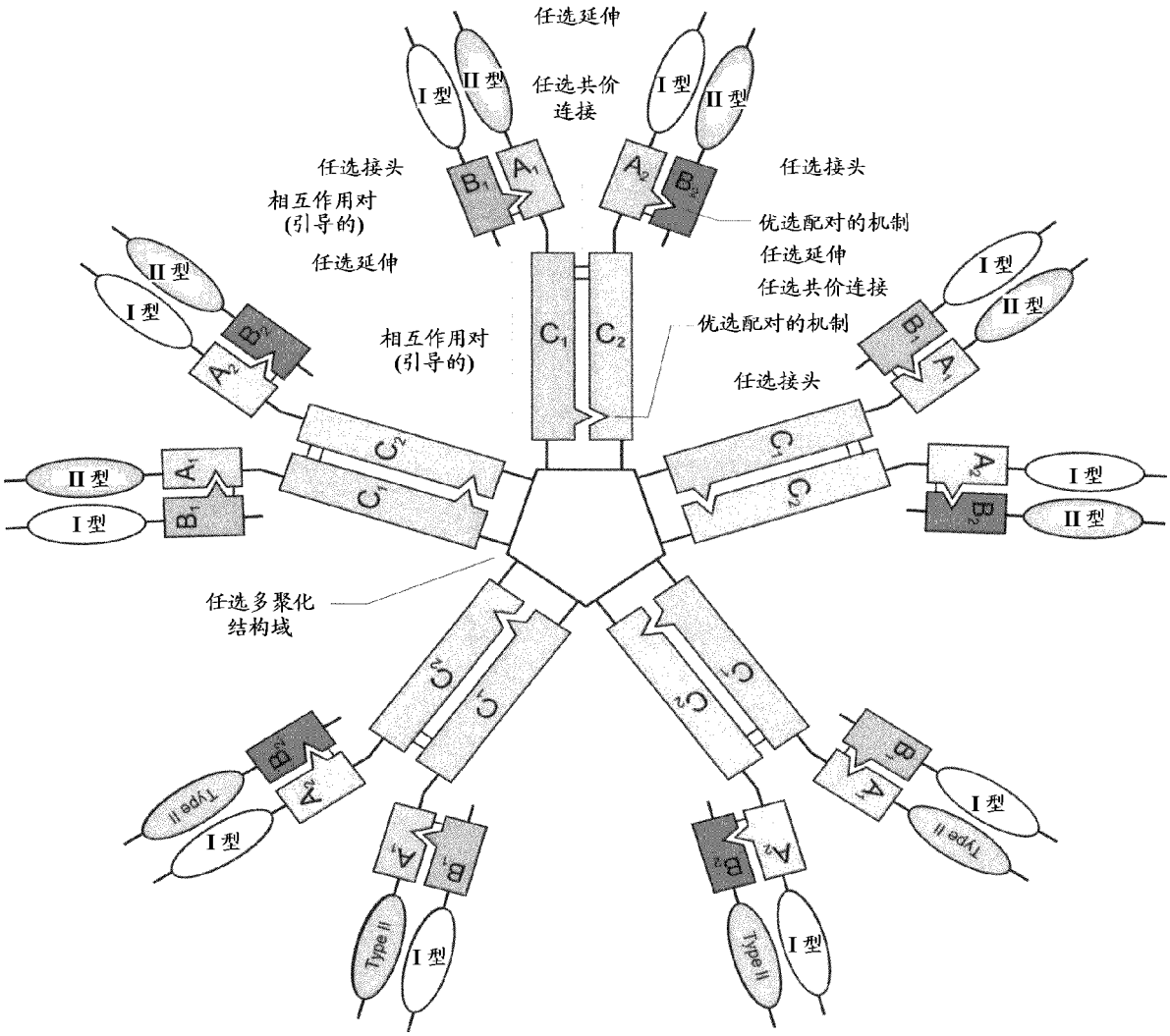

任选延伸
I 型
II 型
任选共价连接
任选接头
相互作用对(引导的)
优选配对的机制
任选延伸
任选共价连接
相互作用对(引导的)
优选配对的机制
任选接头
任选多聚化结构域
Type II

图 16F

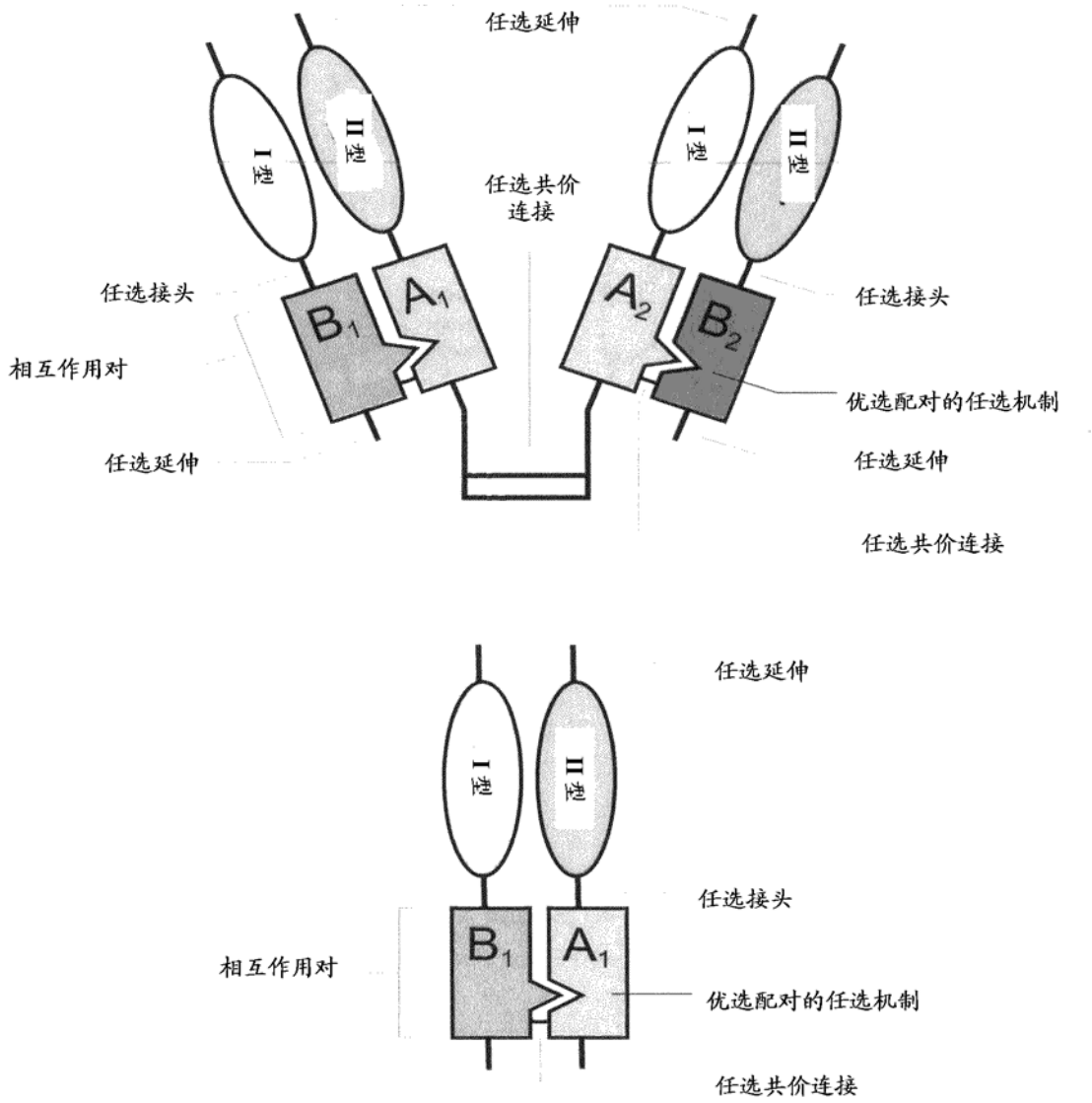

任选延伸
I型
II型
任选共价
连接
任选接头
相互作用对
任选延伸
B_1
A_1
A_2
B_2
任选接头
优选配对的任选机制
任选延伸
任选共价连接
任选延伸
I型
II型
任选接头
相互作用对
B_1
A_1
优选配对的任选机制
任选共价连接

图 16G

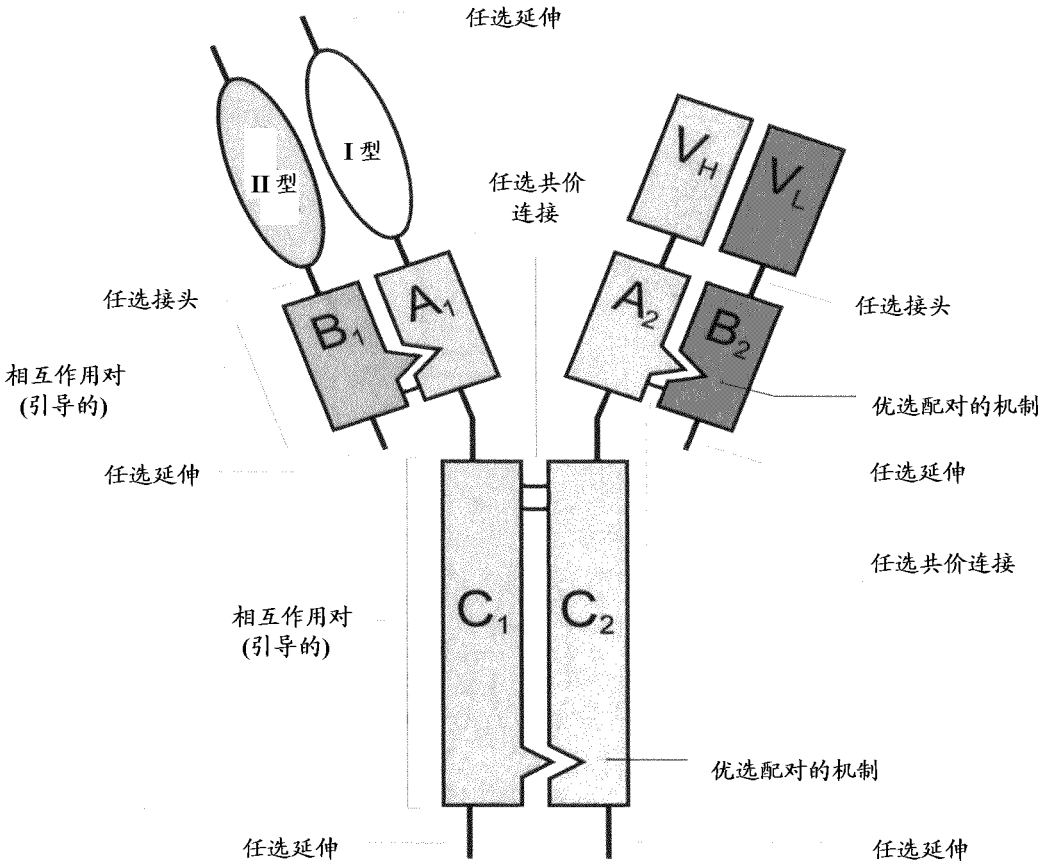

任选延伸
I 型
II 型
任选共价
连接
V_H
V_L
任选接头
B_1
A_1
A_2
B_2
任选接头
相互作用对
(引导的)
优选配对的机制
任选延伸
任选延伸
任选共价连接
相互作用对
(引导的)
C_1
C_2
优选配对的机制
任选延伸
任选延伸

图 17A

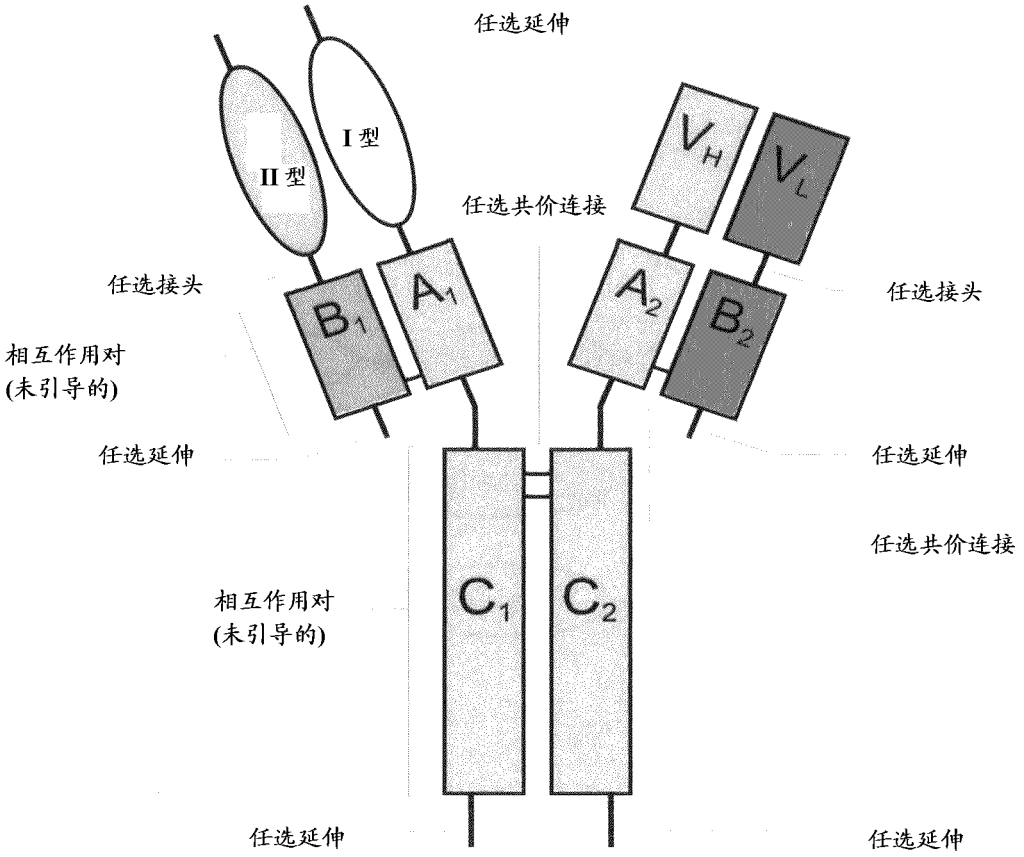

任选延伸
I 型
II 型
任选共价连接
任选接头
任选接头
相互作用对
(未引导的)
任选延伸
任选延伸
任选共价连接
相互作用对
(未引导的)
任选延伸
任选延伸

图 17B

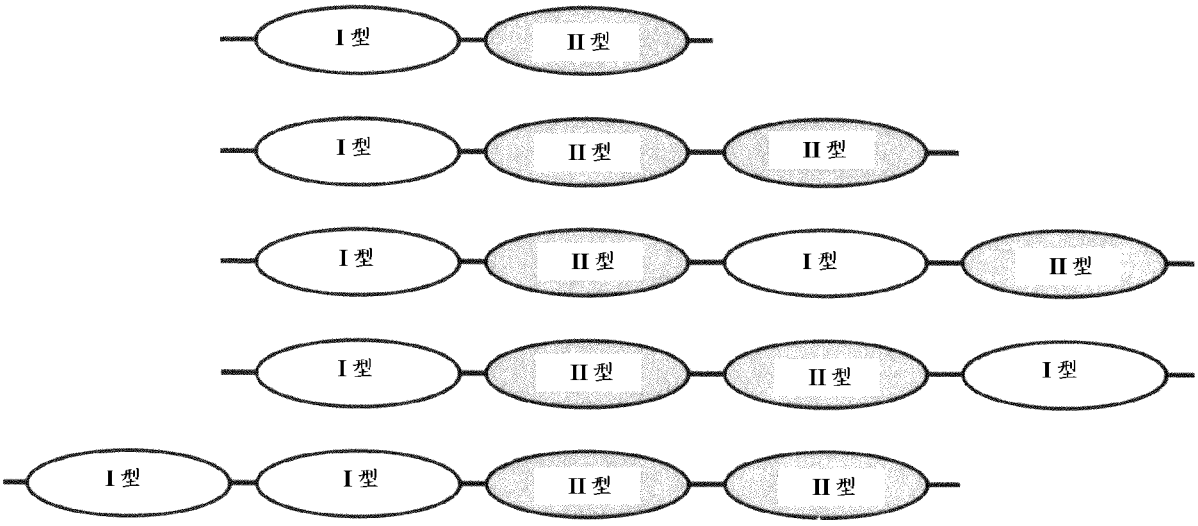

I 型
II 型
I 型
II 型
II 型
I 型
II 型
I 型
II 型
I 型
II 型
II 型
I 型
I 型
II 型
II 型

图 18

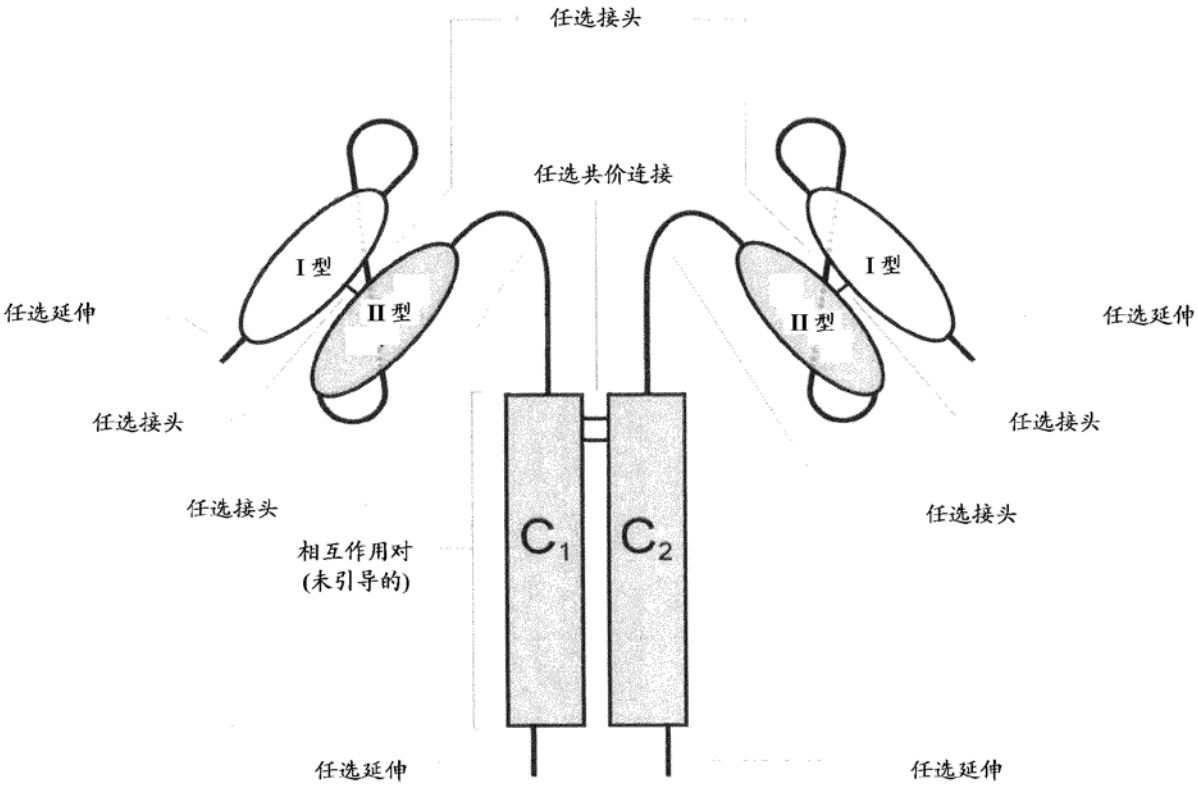

任选接头
任选共价连接
I 型
II 型
任选延伸
任选接头
任选接头
相互作用对
(未引导的)
C1
C2
任选延伸

图 19A

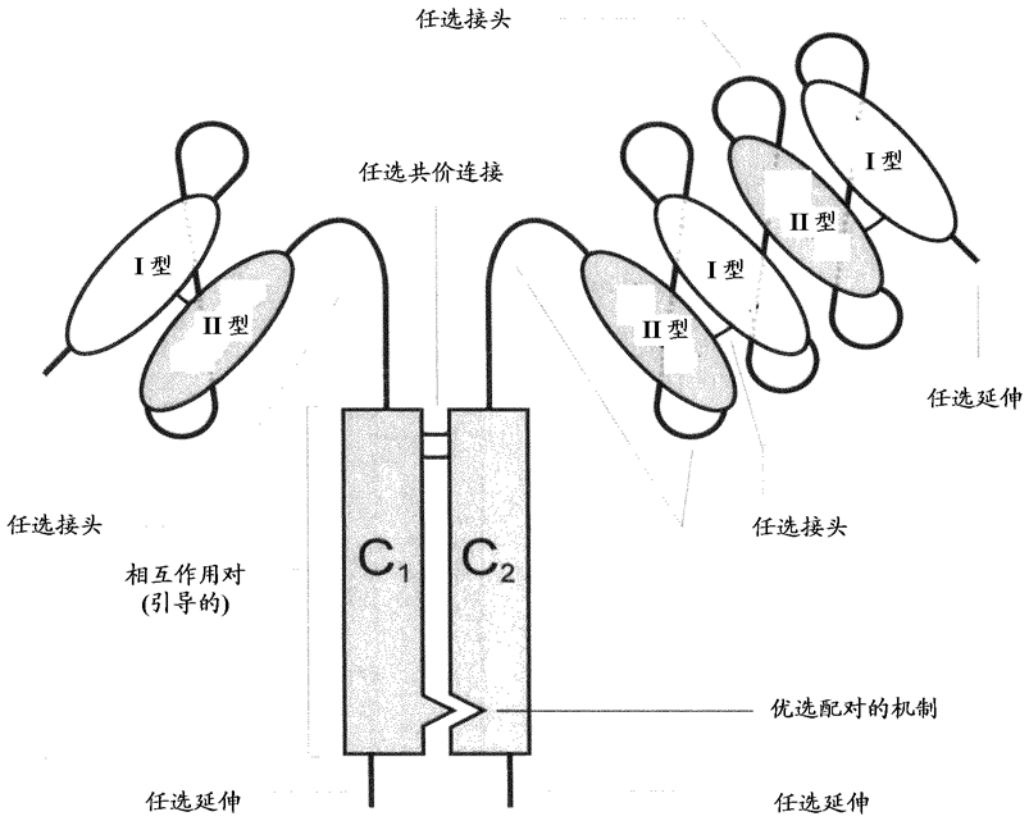

任选接头
任选共价连接
I 型
II 型
任选延伸
任选接头
相互作用对
(引导的)
C1
C2
优选配对的机制
任选延伸

图 19B

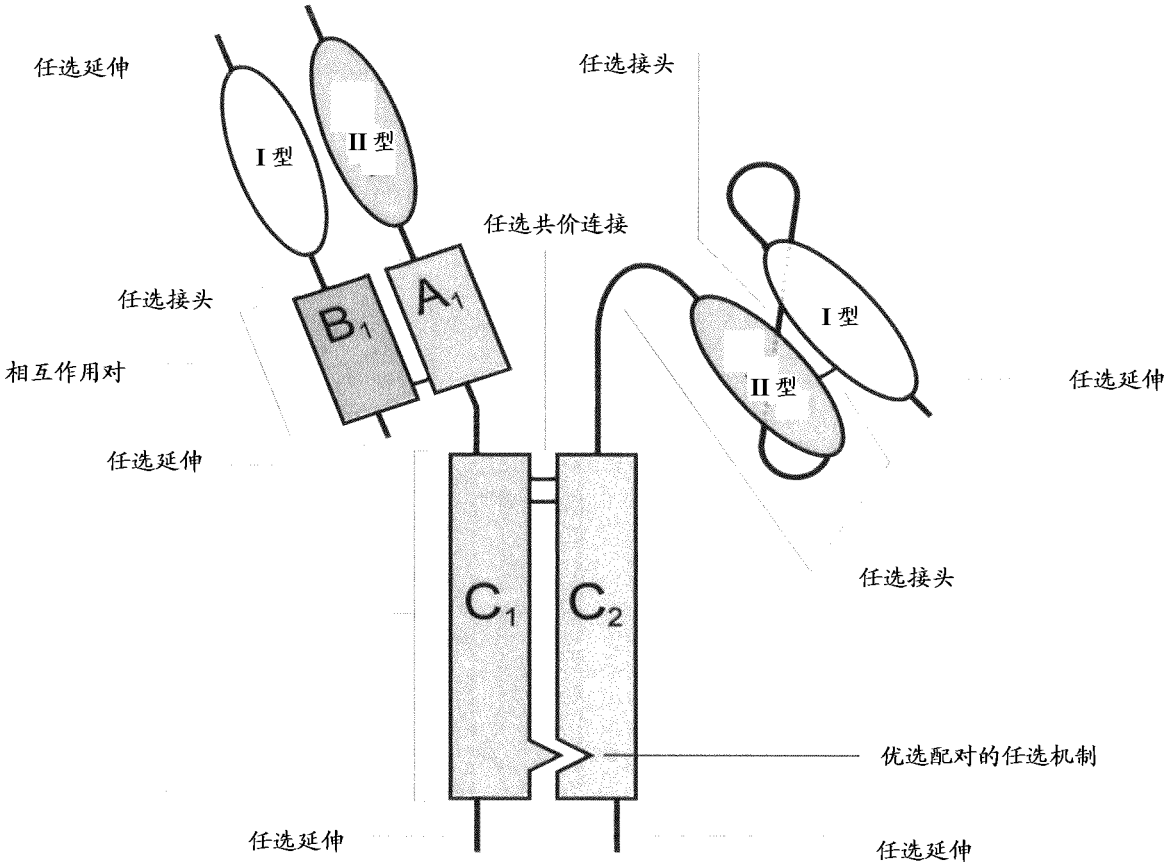

任选延伸
I 型
II 型
任选接头
任选共价连接
任选接头
相互作用对
任选延伸
任选延伸
I 型
II 型
任选延伸
任选接头
优选配对的任选机制
任选延伸
任选延伸

图 19C

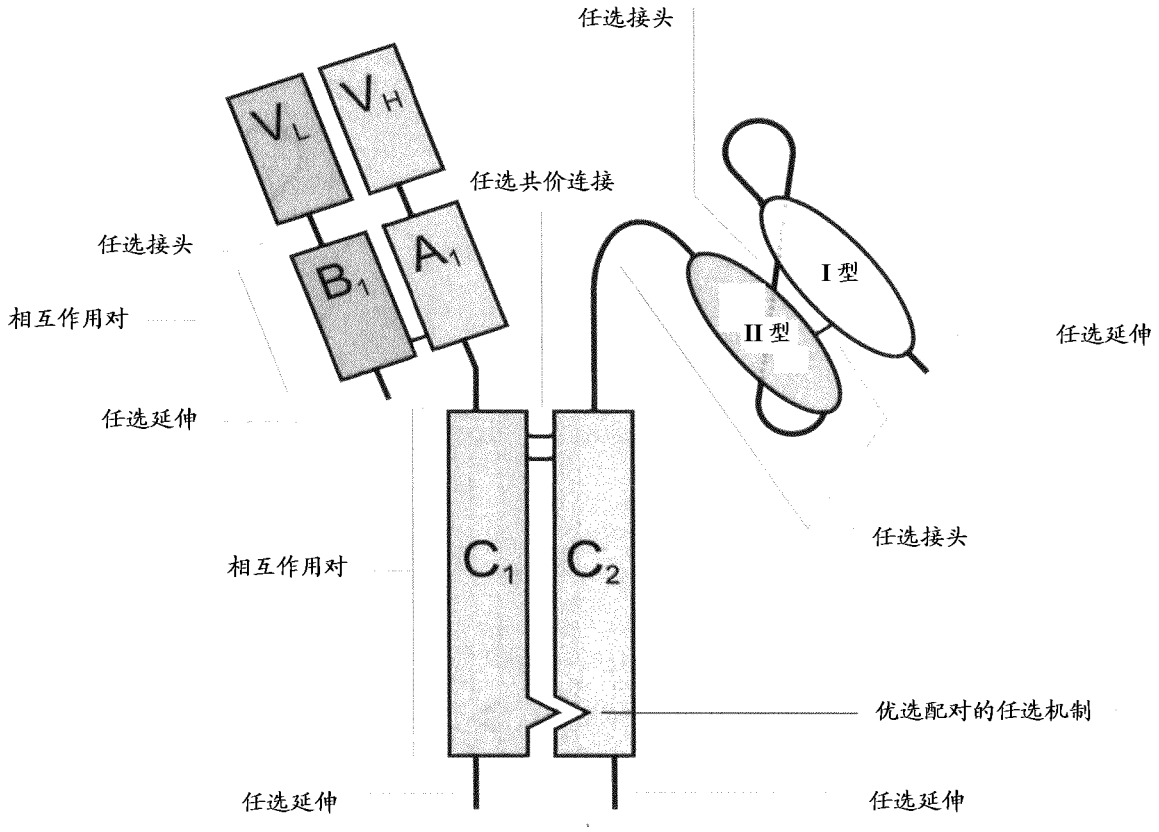

任选接头
V_L
V_H
任选共价连接
任选接头
B_1
A_1
I 型
II 型
相互作用对
任选延伸
任选延伸
任选接头
相互作用对
C_1
C_2
优选配对的任选机制
任选延伸
任选延伸

图 19D