(12) STANDARD PATENT (11) Application No. **AU 2017338916 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Variant ActRIIB proteins and uses thereof

(51) International Patent Classification(s)
C07K 14/71 (2006.01) ***A61P 7/06*** (2006.01)
A61K 38/17 (2006.01) ***C07K 16/46*** (2006.01)

(21) Application No: **2017338916** (22) Date of Filing: **2017.10.05**

(87) WIPO No: **WO18/067874**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/404,718	**2016.10.05**	**US**

(43) Publication Date: **2018.04.12**
(44) Accepted Journal Date: **2022.03.24**

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(56) Related Art
WO 2015/192111 A1
WO 2015/143403 A1
WO 2015/161220 A1
WO 2013/059347 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 April 2018 (12.04.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/067874 A1

(51) International Patent Classification:
C07K 14/71 (2006.01) *A61K 38/17* (2006.01)
C07K 16/46 (2006.01) *A61P 7/06* (2006.01)

(21) International Application Number:
PCT/US2017/055421

(22) International Filing Date:
05 October 2017 (05.10.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/404,718 05 October 2016 (05.10.2016) US

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(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, 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))*

WO 2018/067874 A1

(54) Title: VARIANT ACTRIIB PROTEINS AND USES THEREOF

(57) Abstract: In certain aspects, the present invention provides compositions and methods for modulating (promoting or inhibiting) growth of red blood cells or a tissue, such as bone, cartilage, muscle, fat, and/or neuronal tissue. The present invention also provides methods of screening compounds that modulate activity of an ActRIIB protein and/or an ActRIIB ligand. The compositions and methods provided herein are useful in treating diseases associated with abnormal activity of an ActRIIB protein and/or an ActRIIB ligand.

VARIANT ACTRIIB PROTEINS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority from U.S. Provisional Application No. 62/404,718, filed October 5, 2016. The specification of the foregoing application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

The transforming growth factor-beta (TGF-beta) superfamily contains a variety of growth factors that share common sequence elements and structural motifs. These proteins are known to exert biological effects on a large variety of cell types in both vertebrates and invertebrates. Members of the superfamily perform important functions during embryonic development in pattern formation and tissue specification and can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. The family is divided into two general branches: the BMP/GDF and the TGF-beta/Activin/BMP10 branches, whose members have diverse, often complementary 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., Nat Genet. 1997, 17(1):71-4. Furthermore, in humans, inactive alleles of GDF8 are associated with increased muscle mass and, reportedly, exceptional strength. Schuelke et al., N Engl J Med 2004, 350:2682-8.

Changes in red blood cell levels, bone, cartilage and other tissues may be achieved by agonizing or antagonizing signaling that is mediated by an appropriate TGF-beta family member. Thus, there is a need for agents that function as potent regulators of TGF-beta signaling.

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

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

SUMMARY OF THE INVENTION

In one aspect, the present disclosure provides a variant ActRIIB polypeptide comprising an amino acid sequence that is at least 85% identical to an amino acid sequence that begins at any one of amino acids 20-29 of SEQ ID NO: 2 and ends at any one of amino acids 109-134 of SEQ ID NO: 2, and wherein the polypeptide comprises a lysine at the position corresponding to position 82 of SEQ ID NO: 2.

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

In certain aspects, the present disclosure provides variant ActRIIB polypeptides, particularly variant ActRIIB homomultimer proteins and variant ActRIIB heteromultimer

proteins. As demonstrated by the examples, several variant ActRIIB polypeptides have been identified that display altered binding affinity for one or more ActRIIB-binding ligands. ActRIIB variants that decrease and increase ligand-binding activities were identified. Such variants may be particularly useful for increasing or decreasing ligand selectively compared to a corresponding unmodified ActRIIB polypeptide in a variety of applications. For example, the examples further demonstrate that some of the variant ActRIIB polypeptides have various in vivo effects including, for example, the ability to increase body mass (e.g., muscle mass) as well as increasing red blood cell and hemoglobin levels. Therefore, variant ActRIIB polypeptides should be useful in a variety of therapeutic applications including, for example, those described herein.

In certain aspects, the disclosure relate to variant ActRIIB polypeptides comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acid 20-29 (e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 2 and ends at any one of amino acid 109-134 (e.g., 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 2, and wherein the polypeptide comprises one or more amino acid substitutions at a position of SEQ ID NO: 2 selected from the group consisting of: K55, F82, L79, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 as well as heteromultimer complexes comprising one or more such ActRIIB polypeptides. In some embodiments, the ActRIIB polypeptide comprises an amino acid sequence that is at least 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: 2. In some embodiments, the ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 2. In some embodiments, the ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 20-134 of SEQ ID NO: 2. In some embodiments, the ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 53. In some embodiments, the ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the ActRIIB

polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 5. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to K55 of SEQ ID NO: 2. For example, in some embodiments, the substitution is K55A. In some embodiments, the substitution is K55E. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to L79 of SEQ ID NO: 2. For example, in some embodiments, the substitution is L79D. In some embodiments, the substitution is L79E. In some embodiments, the substitution is L79P. In some embodiments, the substitution is L79A. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to F82 of SEQ ID NO: 2. For example, in some embodiments, the substitution is F82I. In some embodiments, the substitution is F82K. In some embodiments, the substitution is F82A. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to A24 of SEQ ID NO: 2. For example, in some embodiments, the substitution is A24N. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to K74 of SEQ ID NO: 2. For example, in some embodiments, the substitution is K74A. In some embodiments, the substitution is K74A. In some embodiments, the substitution is K74F. In some embodiments, the substitution is K74A. In some embodiments, the substitution is K74I. In some embodiments, the substitution is K74A. In some embodiments, the substitution is K74Y. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to D80 of SEQ ID NO: 2. For example, in some embodiments, the substitution is D80A. In some embodiments, the substitution is D80F. In some embodiments, the substitution is D80K. In some embodiments, the substitution is D80G. In some embodiments, the substitution is D80M. In some embodiments, the substitution is D80I. In some embodiments, the substitution is D80N. In some embodiments, the substitution is D80R. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to R64 of SEQ ID NO: 2. For example, in some embodiments, the substitution is R64K. In some embodiments, the substitution is R64N. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to P129 of SEQ ID NO: 2. For example, in some embodiments, the substitution is P129S. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to P130 of SEQ ID NO: 2. For

example, in some embodiments, the substitution is P130A. In some embodiments, the substitution is P130R. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to E37 of SEQ ID NO: 2. For example, in some embodiments, the substitution is E37A. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to R40 of SEQ ID NO: 2. For example, in some embodiments, the substitution is R40A. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to D54 of SEQ ID NO: 2. For example, in some embodiments, the substitution is D54A. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to R56 of SEQ ID NO: 2. For example, in some embodiments, the substitution is R56A. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to W78 of SEQ ID NO: 2. For example, in some embodiments, the substitution is W78A.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 31. In some embodiments, the variant ActRIIB polypeptide comprises an alanine at the position corresponding to K55 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 33. In some embodiments, the variant ActRIIB polypeptide comprises an alanine at the position corresponding to K55 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 34. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to K55 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 36. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to K55 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 37. In some embodiments, the variant ActRIIB polypeptide comprises an isoleucine at the position corresponding to F82 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 39. In some embodiments, the variant ActRIIB polypeptide comprises an isoleucine at the position corresponding to F82 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 40. In some embodiments, the variant ActRIIB polypeptide comprises a lysine at the position corresponding to F82 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 42. In some embodiments, the variant ActRIIB polypeptide comprises a lysine at the position corresponding to F82 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 43. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to L79 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 45. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to L79 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 46. In some

embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to L79 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to L79 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 49. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to L79 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 50. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to L79 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 51. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to L79 of SEQ ID NO: 2.

In certain aspects, the disclosure relates to a variant ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 52. In some embodiments, the variant ActRIIB polypeptide comprises a glutamic acid at the position corresponding to L79 of SEQ ID NO: 2.

In certain aspects, variant ActRIIB polypeptide of the disclosure form homodimers. In some embodiments, variant ActRIIB polypeptides may from heterodimers through covalent interactions. In some embodiments, variant ActRIIB polypeptides may from heterodimers through non-covalent interactions. In some embodiments, variant ActRIIB polypeptides may from heterodimers through both covalent and non-covalent interactions.

In certain aspects, a variant ActRIIB polypeptide, including homomultimers thereof (e.g., homodimers), binds to one or more TGF-beta superfamily ligands. In some

embodiments, variant ActRIIB polypeptide, including homomultimers thereof, binds to one or more TGF-beta superfamily ligands with a K_D of at least 1 x 10^{-7} M. In some embodiments, the one or more TGF-beta superfamily ligands is selected from the group consisting of: BMP6, BMP7, BMP9, BMP10, GDF3, GDF7, GDF8, GDF11, GDF15, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, and activin BE.

In certain aspects, a variant ActRIIB polypeptide, including homomultimers thereof (e.g., homodimers), inhibits one or more TGF-beta super family ligands. In some embodiments, variant ActRIIB polypeptide, including homomultimers thereof, inhibits signaling of one or more TGF-beta super family ligands. In some embodiments, variant ActRIIB polypeptide, including homomultimers thereof, inhibits Smad signaling of one or more TGF-beta super family ligands. In some embodiments, variant ActRIIB polypeptide, including homomultimers thereof, inhibits signaling of one or more TGF-beta super family ligands in a cell-based assay. In some embodiments, variant ActRIIB polypeptide, including homomultimers thereof, inhibits one or more TGF-beta super family ligands selected from the group consisting of: BMP6, BMP7, BMP9, BMP10, GDF3, GDF7, GDF8, GDF11, GDF15, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, and activin BE.

In certain aspects, the disclosure relates to heteromultimers that comprise at least one variant ActRIIB polypeptide (e.g., one or more variant ActRIIB polypeptides described herein). For example, in some embodiments, a heteromultimer protein of the disclosure comprises a first ActRIIB polypeptide and a second ActRIIB polypeptide, wherein the first ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acid 20-29 (e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 2 and ends at any one of amino acid 109-134 (e.g., 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 2 and comprises one or more amino acid substitutions at positions corresponding to SEQ ID NO: 2 amino acids selected from the group consisting of: A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82, wherein the second ActRIIB polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acid 20-29 (e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 2 and ends at any one of amino acid 109-134 (e.g., 109,

110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 2, and wherein the first ActRIIB polypeptide comprises a different amino acid sequence compared to the second ActRIIB polypeptide. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 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: 2. In some embodiments, the second polypeptide comprises an amino acid sequence that is at least 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: 2. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 2. In some embodiments, the second polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 2. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 20-134 of SEQ ID NO: 2. In some embodiments, the second polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 2. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 53. In some embodiments, the second polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 53. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 5. In some embodiments, the second polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 5. In some embodiments, the first polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the second polypeptide comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 12. In some

embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to K55 of SEQ ID NO: 2. For example, in some embodiments, the substitution is K55A. In some embodiments, the substitution is K55E. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to L79 of SEQ ID NO: 2. For example, in some embodiments, the substitution is L79D. In some embodiments, the substitution is L79E. In some embodiments, the substitution is L79P. In some embodiments, the substitution is L79A. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to F82 of SEQ ID NO: 2. For example, in some embodiments, the substitution is F82I. In some embodiments, the substitution is F82K. In some embodiments, the substitution is F82A. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to A24 of SEQ ID NO: 2. For example, in some embodiments, the substitution is A24N. In some embodiments, the polypeptide comprises an amino acid substitution at the amino acid position corresponding to K74 of SEQ ID NO: 2. For example, in some embodiments, the substitution is K74A. In some embodiments, the substitution is K74A. In some embodiments, the substitution is K74F. In some embodiments, the substitution is K74A. In some embodiments, the substitution is K74I. In some embodiments, the substitution is K74A. In some embodiments, the substitution is K74Y. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to D80 of SEQ ID NO: 2. For example, in some embodiments, the substitution is D80A. In some embodiments, the substitution is D80F. In some embodiments, the substitution is D80K. In some embodiments, the substitution is D80G. In some embodiments, the substitution is D80M. In some embodiments, the substitution is D80I. In some embodiments, the substitution is D80N. In some embodiments, the substitution is D80R. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to R64 of SEQ ID NO: 2. For example, in some embodiments, the substitution is R64K. In some embodiments, the substitution is R64N. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to P129 of SEQ ID NO: 2. For example, in some embodiments, the substitution is P129S. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to P130 of SEQ ID NO: 2. For example, in some embodiments, the substitution is P130A. In some embodiments, the substitution is P130R. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid

position corresponding to E37 of SEQ ID NO: 2. For example, in some embodiments, the substitution is E37A. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to R40 of SEQ ID NO: 2. For example, in some embodiments, the substitution is R40A. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to D54 of SEQ ID NO: 2. For example, in some embodiments, the substitution is D54A. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to R56 of SEQ ID NO: 2. For example, in some embodiments, the substitution is R56A. In some embodiments, the first polypeptide comprises an amino acid substitution at the amino acid position corresponding to W78 of SEQ ID NO: 2. For example, in some embodiments, the substitution is W78A. In some embodiments, the second ActRIIB polypeptide comprises one or more amino acid substitutions at a position of SEQ ID NO: 2 selected from the group consisting of: A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82. For example, in some embodiments, the second ActRIIB polypeptide comprises one or more amino acid substitution with respect to the amino acid sequence of SEQ ID NO: 2 selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, K55A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modifications that promote heteromultimer formation. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modifications that inhibit heteromultimer formation. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modifications that promote heteromultimer formation and one or more amino acid modifications that inhibit heteromultimer formation. In some embodiments, heteromultimers of the disclosure are heterodimers.

In certain aspects, an ActRIIB polypeptides of the disclosure, including variant ActRIIB polypeptides, is fusion protein comprising an ActRIIB polypeptide domain and one or more heterologous domains. In some embodiments, an ActRIIB polypeptide is an ActRIIB-Fc fusion protein. In some embodiments, an ActRIIB-Fc fusion protein further comprises a linker domain positioned between the ActRIIB polypeptide domain and the one or more heterologous domains or Fc domain. In some embodiments, the linker domain is selected from: TGGG, TGGGG, SGGGG, GGGGS, GGG, GGGG, SGGG, and GGGGS.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 13, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 13.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 14, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 14.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 15, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 15.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 16, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 16.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 17, and the second ActRIIB-Fc fusion protein

comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 17.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 18, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 19.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 19, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 18.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 20, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 21.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 21, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 20.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the

amino acid sequence of SEQ ID NO: 22, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 23.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 23, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 22.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 22, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 25.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 25, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 24.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 26, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 27.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%,

80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 27, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 26.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 28, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 29. In some embodiments, the first ActRIIB-Fc fusion protein Fc domain comprises a cysteine at amino acid position 132, glutamic acid at amino acid position 138, a tryptophan at amino acid position 144, and a aspartic acid at amino acid position 217. In some embodiments, the second ActRIIB-Fc fusion protein Fc domain comprises a cysteine at amino acid position 127, a serine at amino acid position 144, an alanine at position 146 an arginine at amino acid position 162, an arginine at amino acid position 179, and a valine at amino acid position 185.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the second ActRIIB-Fc fusion protein, wherein the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 28, and the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 29. In some embodiments, the second ActRIIB-Fc fusion protein Fc domain comprises a cysteine at amino acid position 132, glutamic acid at amino acid position 138, a tryptophan at amino acid position 144, and a aspartic acid at amino acid position 217. In some embodiments, the first ActRIIB-Fc fusion protein Fc domain comprises a cysteine at amino acid position 127, a serine at amino acid position 144, an alanine at position 146 an arginine at amino acid position 162, an arginine at amino acid position 179, and a valine at amino acid position 185.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the second ActRIIB-Fc fusion protein, wherein the first ActRIIB-Fc fusion protein

comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 30, and the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 23. In some embodiments, the first ActRIIB-Fc fusion protein Fc domain comprises a cysteine at amino acid position 132, a tryptophan at amino acid position 144, and a arginine at amino acid position 435. In some embodiments, the second ActRIIB-Fc fusion protein Fc domain comprises cysteine at amino acid position 127, a serine at amino acid position 144, an alanine at amino acid position 146, and a valine at amino acid position 185.

In certain aspects, the disclosure relates to ActRIIB heteromultimer proteins comprising a first ActRIIB-Fc fusion protein and a second ActRIIB-Fc fusion protein wherein the second ActRIIB-Fc fusion protein, wherein the second ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 30, and the first ActRIIB-Fc fusion protein comprises an Fc domain that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 23. In some embodiments, the second ActRIIB-Fc fusion protein Fc domain comprises a cysteine at amino acid position 132, a tryptophan at amino acid position 144, and a arginine at amino acid position 435. In some embodiments, the first ActRIIB-Fc fusion protein Fc domain comprises cysteine at amino acid position 127, a serine at amino acid position 144, an alanine at amino acid position 146, and a valine at amino acid position 185.

In certain aspects, the disclosure relates to a heteromultimer comprising a first ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 33, and second ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 5, wherein the first ActRIIB polypeptide does not comprise the amino acid sequence of the second ActRIIB polypeptide. In some embodiments, the first ActRIIB polypeptide comprises a alanine at the amino acid position corresponding to 55 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide does not comprise a alanine at the amino acid position corresponding to 55 of SEQ ID NO: 2. In some embodiments, the second ActRIIB

polypeptide comprises a lysine at the amino acid position corresponding to 55 of SEQ ID NO: 2. In some embodiments, the first ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of F82, L79, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A. In some embodiments, the second ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of F82, L79, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that promote heteromultimer formation. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that inhibit heteromultimer formation. In some embodiments, the heteromultimer is a heterodimer.

In certain aspects, the disclosure relates to a heteromultimer comprising a first ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 36, and second ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 5, wherein the first ActRIIB polypeptide does not comprise the amino acid sequence of the second ActRIIB polypeptide. In some embodiments, the first ActRIIB polypeptide comprises a glutamic acid at the amino acid position corresponding to 55 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide does not comprise a glutamic acid at the amino acid position corresponding to 55 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide comprises a lysine at the amino acid position corresponding to 55 of SEQ ID NO: 2. In some embodiments, the first ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of F82, L79, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group

consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A. In some embodiments, the second ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of F82, L79, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that promote heteromultimer formation. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that inhibit heteromultimer formation. In some embodiments, the heteromultimer is a heterodimer.

In certain aspects, the disclosure relates to a heteromultimer comprising a first ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 39, and second ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 5, wherein the first ActRIIB polypeptide does not comprise the amino acid sequence of the second ActRIIB polypeptide. In some embodiments, the first ActRIIB polypeptide comprises a isoleucine at the amino acid position corresponding to 82 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide does not comprise a isoleucine acid at the amino acid position corresponding to 82 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide comprises a phenylalanine at the amino acid position corresponding to 82 of SEQ ID NO: 2. In some embodiments, the first ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of L79, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, and D80 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, and D80R. In some embodiments, the second ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of L79, A24, K74, R64, P129, P130, E37, R40, D54, R56,

W78, and D80 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, and D80R. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that promote heteromultimer formation. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that inhibit heteromultimer formation. In some embodiments, the heteromultimer is a heterodimer.

In certain aspects, the disclosure relates to a heteromultimer comprising a first ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 42, and second ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 5, wherein the first ActRIIB polypeptide does not comprise the amino acid sequence of the second ActRIIB polypeptide. In some embodiments, first ActRIIB polypeptide comprises a lysine at the amino acid position corresponding to 82 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide does not comprise a lysine acid at the amino acid position corresponding to 82 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide comprises a phenylalanine at the amino acid position corresponding to 82 of SEQ ID NO: 2. In some embodiments, the first ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of L79, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, and D80 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, and D80R. In some embodiments, the second ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of L79, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, and D80 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79A, L79D, L79E, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, and D80R. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino

acid modification that promote heteromultimer formation. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that inhibit heteromultimer formation. In some embodiments, the heteromultimer is a heterodimer.

In certain aspects, the disclosure relates to a heteromultimercomprising a first ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 45, and second ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 48, wherein the first ActRIIB polypeptide does not comprise the amino acid sequence of the second ActRIIB polypeptide. In some embodiments, the first ActRIIB polypeptide comprises an acidic amino acid position corresponding to 79 of SEQ ID NO: 2. In some embodiments, the acidic amino acid is an aspartic acid. In some embodiments, the acidic amino acid is a glutamic acid. In some embodiments, the second ActRIIB polypeptide does not comprise an acidic acid (e.g., aspartic acid or glutamic acid) at the amino acid position corresponding to 79 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide comprises a leucine at the amino acid position corresponding to 79 of SEQ ID NO: 2. In some embodiments, the first ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of F82, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A. In some embodiments, the second ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of F82, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that promote heteromultimer formation. In some embodiments, the first ActRIIB polypeptide and/or the second ActRIIB polypeptide comprise one or more amino acid modification that inhibit heteromultimer formation. In some embodiments, the heteromultimer is a heterodimer.

In certain aspects, the disclosure relates to a heteromultimer comprising a first ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 50, and second ActRIIB polypeptide that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 52, wherein the first ActRIIB polypeptide does not comprise the amino acid sequence of the second ActRIIB polypeptide. In some embodiments, the first ActRIIB polypeptide comprises an acidic amino acid position corresponding to 79 of SEQ ID NO: 2. In some embodiments, the acidic amino acid is an aspartic acid. In some embodiments, the acidic amino acid is a glutamic acid. In some embodiments, the second ActRIIB polypeptide does not comprise an acidic acid (e.g., aspartic acid or glutamic acid) at the amino acid position corresponding to 79 of SEQ ID NO: 2. In some embodiments, the second ActRIIB polypeptide comprises a leucine at the amino acid position corresponding to 79 of SEQ ID NO: 2. In some embodiments, the first ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of F82, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, L79P, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A. In some embodiments, the second ActRIIB polypeptide comprises one or more amino acid substitutions at the amino acid positions corresponding to any one of F82, A24, K74, R64, P129, P130, E37, R40, D54, R56, W78, D80, and F82 of SEQ ID NO: 2. In some embodiments, the one or more amino acid substitutions is selected from the group consisting of: A24N, K74A, R64K, R64N, K74A, P129S, P130A, P130R, E37A, R40A, D54A, R56A, K74F, K74I, K74Y, W78A, D80A, D80F, D80G, D80I, D80K, D80M, D80M, D80N, D80R, and F82A.

In certain embodiments, the disclosure provides for a heteromultimer protein comprising any of the ActRIIB polypeptides disclosed herein and a second polypeptide selected from the group consisting of: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, TGFBRII, BMPRII, and MISRII polypeptide, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK1 polypeptide or a functional fragment thereof. In some embodiments, the ALK1 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID

NO: 54, or functional fragments thereof. In some embodiments, the ALK1 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 54, 55, 56, 57, 60, and 61, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK2 polypeptide or a functional fragment thereof. In some embodiments, the ALK2 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 64 or 65, or functional fragments thereof. In some embodiments, the ALK2 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID Nos: 64, 65, 66, 67, 70, and 71, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK3 polypeptide or a functional fragment thereof. In some embodiments, the ALK3 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 74, or functional fragments thereof. In some embodiments, the ALK3 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 74, 75, 76, 77, 80, or 81, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK4 polypeptide or a functional fragment thereof. In some embodiments, the ALK4 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 84 or 85, or functional fragments thereof. In some embodiments, the ALK4 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 84, 86, 85, 87, 88, 89, 92, and 93, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK5 polypeptide or a functional fragment thereof. In some embodiments, the ALK5 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96 or 97, or functional fragments thereof. In some embodiments, the ALK5 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%,

98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96, 98, 97, 99, 100, 101, 104, and 105, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK6 polypeptide or a functional fragment thereof. In some embodiments, the ALK6 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 108 or 110, or functional fragments thereof. In some embodiments, the ALK6 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 108, 109, 110, 111, 112, 113, 116, and 117, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK7 polypeptide or a functional fragment thereof. In some embodiments, the ALK7 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 120, 121, or 122, or functional fragments thereof. In some embodiments, the ALK7 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 120, 123, 124, 125, 121, 126, 122, 127, 128, 129, 130, 133, and 134, or functional fragments thereof. In some embodiments, the second polypeptide is an ActRIIA polypeptide or a functional fragment thereof. In some embodiments, the ActRIIA polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 137, or functional fragments thereof. In some embodiments, the ActRIIA polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 137, 138, 139, 140, 141, 144, and 145, or functional fragments thereof. In some embodiments, the second polypeptide is an TGFBRII polypeptide or a functional fragment thereof. In some embodiments, the TGFBRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 204, or functional fragments thereof. In some embodiments, the TGFBRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 161, 162, 160, 163, 164, 165, 166, 167, 172, 173, 174, and 175, or functional fragments thereof. In some embodiments, the second polypeptide is an

BMPRII polypeptide or a functional fragment thereof. In some embodiments, the BMPRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 148 or 149, or functional fragments thereof. In some embodiments, the BMPRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 148, 150, 149, 151, 152, 153, 156, and 157, or functional fragments thereof. In some embodiments, the second polypeptide is an MISRII polypeptide or a functional fragment thereof. In some embodiments, the MISRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 180, 181, or 182, or functional fragments thereof. In some embodiments, the MISRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 180, 183, 181, 184, 182, and 185, or functional fragments thereof.

In certain aspects, heteromultimers of the disclosure bind to one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure binds to one or more TGF-beta superfamily ligands with a K_D of at least 1 x 10^{-7} M. In some embodiments, the one or more TGF-beta superfamily ligands is selected from the group consisting of: BMP6, BMP7, BMP9, BMP10, GDF3, GDF7, GDF8, GDF11, GDF15, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, and activin BE.

In certain aspects, heteromultimers of the disclosure inhibits one or more TGF-beta super family ligands. In some embodiments, heteromultimers of the disclosure inhibits signaling of one or more TGF-beta super family ligands. In some embodiments, heteromultimers of the disclosure inhibits Smad signaling of one or more TGF-beta super family ligands. In some embodiments, heteromultimers of the disclosure inhibits signaling of one or more TGF-beta super family ligands in a cell-based assay. In some embodiments, heteromultimers of the disclosure inhibits one or more TGF-beta super family ligands selected from the group consisting of: BMP6, BMP7, BMP9, BMP10, GDF3, GDF7, GDF8, GDF11, GDF15, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, and activin BE.

In certain aspects, the disclosure relates to ActRIIB polypeptides, including variant ActRIIB polypeptides as well as homomultimer and heteromultimers comprising the same, that comprises one or more amino acid modifications selected from the group consisting of: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, and an amino acid conjugated to a lipid moiety. In some embodiments, ActRIIB polypeptides of the disclosure are glycosylated and has a glycosylation pattern obtainable from of the polypeptide in a CHO cell.

In certain embodiments, the disclosure provides for a heteromultimer protein comprising any of the ActRIIB polypeptides disclosued herein and a second polypeptide selected from the group consisting of: ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, ALK7, ActRIIA, TGFBRII, BMPRII, and MISRII polypeptide, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK1 polypeptide or a functional fragment thereof. In some embodiments, the ALK1 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 54, or functional fragments thereof. In some embodiments, the ALK1 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK2 polypeptide or a functional fragment thereof. In some embodiments, the ALK2 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 64 or 65, or functional fragments thereof. In some embodiments, the ALK2 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID Nos: 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK3 polypeptide or a functional fragment thereof. In some embodiments, the ALK3 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 74, or functional fragments thereof. In some embodiments, the ALK3 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%,

97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 74, 75, 76, 77, 80, or 81, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK4 polypeptide or a functional fragment thereof. In some embodiments, the ALK4 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 84 or 85, or functional fragments thereof. In some embodiments, the ALK4 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 84, 86, 85, 87, 88, 89, 90, 91, 92, 93, 94, and 95, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK5 polypeptide or a functional fragment thereof. In some embodiments, the ALK5 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96 or 97, or functional fragments thereof. In some embodiments, the ALK5 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96, 98, 97, 99, 100, 101, 102, 103, 104, 105, 106, and 107, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK6 polypeptide or a functional fragment thereof. In some embodiments, the ALK6 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 108 or 110, or functional fragments thereof. In some embodiments, the ALK6 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, and 119, or functional fragments thereof. In some embodiments, the second polypeptide is an ALK7 polypeptide or a functional fragment thereof. In some embodiments, the ALK7 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 120, 121, or 122, or functional fragments thereof. In some embodiments, the ALK7 polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 120, 123, 124, 125, 121, 126, 122, 127, 128, 129, 130, 131, 132, 133, 134, 135, and 136, or functional fragments thereof. In some embodiments, the second

polypeptide is an ActRIIA polypeptide or a functional fragment thereof. In some embodiments, the ActRIIA polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 137, or functional fragments thereof. In some embodiments, the ActRIIA polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, and 147, or functional fragments thereof. In some embodiments, the second polypeptide is an TGFBRII polypeptide or a functional fragment thereof. In some embodiments, the TGFBRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 204, or functional fragments thereof. In some embodiments, the TGFBRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 161, 162, 160, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, and 179, or functional fragments thereof. In some embodiments, the second polypeptide is an BMPRII polypeptide or a functional fragment thereof. In some embodiments, the BMPRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 148 or 149, or functional fragments thereof. In some embodiments, the BMPRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 148, 150, 149, 151, 152, 153, 154, 155, 156, 157, 158, and 159, or functional fragments thereof. In some embodiments, the second polypeptide is an MISRII polypeptide or a functional fragment thereof. In some embodiments, the MISRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 180, 181, or 182, or functional fragments thereof. In some embodiments, the MISRII polypeptide or functional fragment thereof comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 180, 183, 181, 184, 182, 185, 186, 187, 188, 189, 190, 191, 192, and 193, or functional fragments thereof.

In certain aspects, the disclosure relates to pharmaceutical preparations comprising a ActRIIB polypeptide, including variant ActRIIB polypeptides as well as homomultimer and heteromultimers comprising the same, a pharmaceutically acceptable carrier. In some embodiments, pharmaceutical preparations comprising one or more ActRIIB heteromulitmers comprises less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% homomultimers.

In certain aspects, the disclosure relates to isolated and/or recombinant nucleic acids comprising a coding sequence for one or more of the ActRIIB polypeptide(s) as described herein. For example, in some embodiments, the disclosure relates to an isolated and/or recombinant nucleic acid that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleic acid sequence corresponding to any one of SEQ ID Nos: 3, 10, 31, 35, 38, 41, 44, or 47. In some embodiments, an isolated and/or recombinant polynucleotide sequence of the disclosure comprises a promoter sequence operably linked to a coding sequence described herein (e.g., a nucleic acid that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleic acid sequence corresponding to any one of SEQ ID Nos: 3, 10, 31, 35, 38, 41, 44, or 47). In some embodiments, the disclosure relates to vectors comprising an isolated and/or recombinant nucleic acid described herein (e.g., a nucleic acid that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleic acid sequence corresponding to any one of SEQ ID Nos: 3, 10, 31, 35, 38, 41, 44, or 47). In some embodiments, the disclosure relates to a cell comprising an isolated and/or recombinant polynucleotide sequence described herein (e.g., a nucleic acid that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleic acid sequence corresponding to any one of SEQ ID Nos: 3, 10, 31, 35, 38, 41, 44, or 47). In some embodiments, the cell is a CHO cell. In some embodiments, the cell is a COS cell.

In certain aspects, the disclosure relates to methods of making ActRIIB polypeptides, including variant ActRIIB polypeptides as well as homomultimer and heteromultimers comprising the same, as described herein. Such a method may include expressing any of the nucleic acids) disclosed herein in a suitable cell (e.g., a CHO cell or COS cell). Such a method may comprise: a) culturing a cell under conditions suitable for expression of the soluble ActRIIB polypeptide, wherein said cell comprise with an ActRIIB polypeptide expression construct. In some embodiments, the method further comprises recovering the expressed ActRIIB polypeptide. ActRIIB polypeptides 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 some embodiments, the disclosure relates to methods for increasing red blood cell levels or hemoglobin levels in a patient, comprising administering a patient in need thereof an ActRIIB polypeptide, including variant ActRIIB polypeptides as well as homomultimer and heteromultimers comprising the same, as described herein.

In some embodiments, the disclosure relates to methods for treating anemia or a disorder associated with anemia (e.g., those described herein) in a patient, comprising administering a patient in need thereof an ActRIIB polypeptide, including variant ActRIIB polypeptides as well as homomultimer and heteromultimers comprising the same, as described herein.

In some embodiments, the disclosure relates to methods for increasing muscle mass and/or muscle strength in a patient, comprising administering a patient in need thereof an ActRIIB polypeptide, including variant ActRIIB polypeptides as well as homomultimer and heteromultimers comprising the same, as described herein.

In some embodiments, the disclosure relate to methods for treating a muscle-related disorder in a patient, comprising administering a patient in need thereof an ActRIIB, including variant ActRIIB polypeptides as well as homomultimer and heteromultimers comprising the same, as described herein. In some embodiments, the disorder is associated with undesirably low muscle growth and/or muscle weakness. Such disorders include muscle atrophy, muscular dystrophy, amyotrophic lateral sclerosis (ALS), and a muscle wasting disorder (e.g., cachexia, anorexia, DMD syndrome, BMD syndrome, AIDS wasting syndrome, muscular dystrophies, neuromuscular diseases, motor neuron diseases, diseases of the neuromuscular junction, and inflammatory myopathies).

In some embodiments, the disclosure relate to methods for decreasing the body fat content or reducing the rate of increase in body fat content, and for treating a disorder associated with undesirable body weight gain, such as obesity, non-insulin dependent diabetes mellitus (NIDDM), cardiovascular disease, cancer, hypertension, osteoarthritis, stroke, respiratory problems, and gall bladder disease, comprising administering a patient in need thereof an ActRIIB, including variant ActRIIB polypeptides as well as homomultimer and heteromultimers comprising the same, as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows schematic examples of heteromeric protein complexes comprising a first variant ActRIIB polypeptide (indicated as "X") and either a second variant ActRIIB polypeptide (indicated as "Y") or an unmodified ActRIIB polypeptide (indicated as "Y") In the illustrated embodiments, the first variant ActRIIB polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"), and either a second variant ActRIIB polypeptide or an unmodified ActRIIB polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"). Suitable interaction pairs include, 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 first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB 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 1A. 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 1B.

Figure 2 shows an alignment of extracellular domains of human ActRIIA and human ActRIIB with the residues that are deduced herein to directly contact ligand (indicated by boxes) based on composite analysis of multiple ActRIIB and ActRIIA crystal structures.

Figure 3 shows a multiple sequence alignment of various vertebrate ActRIIB precursor proteins without their intracellular domains, human ActRIIA precursor protein without its intracellular domain, and a consensus ActRII precursor protein.

Figure 4 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 (SEQ ID NO: 13) Fc to promote asymmetric chain pairing and the corresponding positions with respect to other isotypes IgG4 (SEQ ID NO: 17), IgG2 (SEQ ID NO: 14), and IgG3 (SEQ ID NO: 15).

Figure 5 shows the amino acid sequence of a human ActRIIB extracellular domain polypeptide (SEQ ID NO: 1) in which numbering is based on the native human ActRIIB precursor sequence (see SEQ ID NO: 2).

Figure 6 shows the amino acid sequence of human ActRIIB precursor protein (SEQ ID NO: 2; NCBI Reference Sequence NP_001097.2). The signal peptide is underlined, the extracellular domain is in bold (also referred to as SEQ ID NO: 1), and the potential N-linked glycosylation sites are boxed.

Figure 7 shows a nucleic acid sequence encoding a human ActRIIB(20-134) extracellular domain polypeptide.

Figure 8 shows a nucleic acid sequence encoding human ActRIIB precursor protein. SEQ ID NO: 4 consists of nucleotides 25-1560 of NCBI Reference Sequence NM_001106.

Figure 9 shows values for ligand binding kinetics of homodimeric Fc-fusion proteins comprising variant or unmodified ActRIIB domains, as determined by surface plasmon resonance at 37°C. Amino acid numbering is based on SEQ ID NO: 2.

Figure 10 shows values for ligand binding kinetics of homodimeric Fc-fusion proteins comprising variant or unmodified ActRIIB domains, as determined by surface plasmon resonance at 25°C. Amino acid numbering is based on SEQ ID NO: 2.

Figure 11 shows changes in body weight from baseline for wild-type mice treated with vehicle or homodimeric Fc-fusion proteins comprising variant or unmodified ActRIIB domains.

Figure 12 shows hemoglobin concentrations in wild-type mice treated with vehicle or homodimeric Fc-fusion proteins comprising variant or unmodified ActRIIB domains.

Figure 13 shows red blood cell counts in cynomolgus monkeys treated with ActRIIB-Fc, ActRIIA-Fc, or ActRIIB(F82I)-Fc at 9 mg/kg (s.c.) on days 1 and 15. ActRIIB(F82I)-Fc treatment increased RBC counts compared to ActRIIB-Fc (negative control) by an amount similar to that of ActRIIA-Fc (postitive control).

DETAILED DESCRIPTION

1. Overview

In certain aspects, the present invention relates to ActRIIB polypeptides. As used herein, the term "ActRIIB" refers to a family of activin receptor type IIB (ActRIIB) proteins and ActRIIB-related proteins, derived from any species. Members of the ActRIIB family are generally all 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. The amino acid sequence of human ActRIIB precursor protein is shown in Figure 6 (SEQ ID NO: 2).

The term "ActRIIB polypeptide" is used to refer to 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. For example, ActRIIB polypeptides include polypeptides derived from the sequence of any known ActRIIB having a sequence at least about 80% identical to the sequence of an ActRIIB polypeptide, and preferably at least 85%, 90%, 95%, 97%, 99% or greater identity.

In a specific embodiment, the invention relates to soluble ActRIIB polypeptides. As described herein, the term "soluble ActRIIB polypeptide" generally refers to polypeptides comprising an extracellular domain of an ActRIIB protein. The term "soluble ActRIIB polypeptide," as used herein, includes any naturally occurring extracellular domain of an ActRIIB protein as well as any variants thereof (including mutants, fragments and peptidomimetic forms) that retain a useful activity. For example, the extracellular domain of an ActRIIB protein binds to a ligand and is generally soluble. Examples of soluble ActRIIB polypeptides include an ActRIIB extracellular domain (SEQ ID NO: 1) shown in Figure 5 as well as SEQ ID NO: 53. Other examples of soluble ActRIIB polypeptides comprise a signal sequence in addition to the extracellular domain of an ActRIIB protein (see Example 1). The signal sequence can be a native signal sequence of an ActRIIB, or a signal sequence from another protein, such as a tissue plasminogen activator (TPA) signal sequence or a honey bee melatin signal sequence.

TGF-β signals are mediated by heteromeric complexes of type I and type II serine/ threonine kinase receptors, which phosphorylate and activate downstream Smad proteins upon ligand stimulation (Massagué, 2000, Nat. Rev. Mol. Cell Biol. 1:169-178). These type I and type II receptors are all transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine specificity. Type I receptors are essential for signaling, and type II receptors are required for binding ligands. Type I and type II activin receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors.

Two related type II receptors, ActRIIA and ActRIIB, have been identified as the type II receptors for activins (Mathews and Vale, 1991, Cell 65:973-982; Attisano et al., 1992, Cell 68: 97-108). Besides activins, ActRIIA and ActRIIB can biochemically interact with several other TGF-β family proteins, including BMP7, Nodal, GDF8, and GDF11 (Yamashita et al., 1995, J. Cell Biol. 130:217-226; Lee and McPherron, 2001, Proc. Natl. Acad. Sci. 98:9306-9311; Yeo and Whitman, 2001, Mol. Cell 7: 949-957; Oh et al., 2002, Genes Dev. 16:2749-54). Applicants have found that soluble ActRIIA-Fc fusion proteins and ActRIIB-Fc fusion proteins have substantially different effects in vivo, with ActRIIA-Fc having primary effects on bone and ActRIIB-Fc having primary effects on skeletal muscle.

In certain embodiments, the present invention relates to antagonizing a ligand of ActRIIB receptors (also referred to as an ActRIIB ligand) with a subject ActRIIB polypeptide (e.g., a soluble ActRIIB polypeptide). Thus, compositions and methods of the present invention are useful for treating disorders associated with abnormal activity of one or more ligands of ActRIIB receptors. Exemplary ligands of ActRIIB receptors include some TGF-β family members, such as activin, Nodal, GDF8, GDF11, and BMP7.

Activins are dimeric polypeptide growth factors and belong to the TGF-beta superfamily. There are three activins (A, B, and AB) that are homo/heterodimers of two closely related β subunits ($\beta_A\beta_A$, $\beta_B\beta_B$, and $\beta_A\beta_B$). 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 (DePaolo et al., 1991, Proc SocEp Biol Med. 198:500-512; Dyson et al., 1997, Curr Biol. 7:81-84; Woodruff, 1998, Biochem Pharmacol. 55:953-963). Moreover, erythroid differentiation factor (EDF) isolated from the stimulated human monocytic leukemic cells was found to be identical to activin A (Murata et al., 1988, PNAS, 85:2434). It was suggested that activin A acts as a natural regulator of erythropoiesis in the bone marrow. In several tissues, activin signaling is antagonized by its related heterodimer, inhibin. For example, during the release of follicle-stimulating hormone (FSH) from the pituitary, activin promotes FSH secretion and synthesis, while inhibin prevents FSH secretion and synthesis. Other proteins that may regulate activin bioactivity and/or bind to activin include follistatin (FS), follistatin-related protein (FSRP), α_2-macroglobulin, Cerberus, and endoglin.

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. Recent studies support the idea that ActRIIA and ActRIIB serve as type II receptors for Nodal (Sakuma et al., Genes Cells. 2002, 7:401-12). It is suggested that Nodal ligands interact with their co-factors (e.g., cripto) 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 both activin-TGF-beta pathway Smads, Smad2 and Smad3. Further evidence has shown that the extracellular cripto protein is required for Nodal signaling, making it distinct from activin or TGF-beta signaling.

Growth and differentiation factor-8 (GDF8) is also known as myostatin. GDF8 is a negative regulator of skeletal muscle mass. GDF8 is highly expressed in the developing and adult skeletal muscle. The GDF8 null mutation in transgenic mice is characterized by a marked hypertrophy and hyperplasia of the skeletal muscle (McPherron et al., Nature, 1997, 387:83-90). Similar increases in skeletal muscle mass are evident in naturally occurring mutations of GDF8 in cattle (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; and Kambadur et al., Genome Res., 1997, 7:910-915) and, strikingly, in humans (Schuelke et al., N Engl J Med 2004;350:2682-8). Studies have also shown that muscle wasting associated with HIV-infection in humans is accompanied by increases in GDF8 protein expression (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 (WO 00/43781). The GDF8 propeptide can noncovalently bind to the mature GDF8 domain dimer, inactivating its biological activity (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 (Gamer et al. (1999) Dev. Biol., 208: 222-232).

Growth and differentiation factor-11 (GDF11), also known as BMP11, is a secreted protein (McPherron et al., 1999, Nat. Genet. 22: 260-264). GDF11 is expressed in the tail bud, limb bud, maxillary and mandibular arches, and dorsal root ganglia during mouse development (Nakashima et al., 1999, Mech. Dev. 80: 185-189). GDF11 plays a unique role in patterning both mesodermal and neural tissues (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 (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 (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).

Bone morphogenetic protein (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 IIB. 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 (Macias-Silva et al., 1998, J Biol Chem. 273:25628-36).

In certain aspects, the present invention relates to the use of certain ActRIIB polypeptides (e.g., soluble ActRIIB polypeptides) to antagonize the signaling of ActRIIB ligands generally, in any process associated with ActRIIB activity. Optionally, ActRIIB polypeptides of the invention may antagonize one or more ligands of ActRIIB receptors, such as activin, Nodal, GDF8, and GDF11, and may therefore be useful in the treatment of additional disorders.

Therefore, the present invention contemplates using ActRIIB polypeptides in treating or preventing diseases or conditions that are associated with abnormal activity of an ActRIIB or an ActRIIB ligand. ActRIIB or ActRIIB ligands are involved in the regulation of many critical biological processes. Due to their key functions in these processes, they may be desirable targets for therapeutic intervention. For example, ActRIIB polypeptides (e.g., soluble ActRIIB polypeptides) may be used to treat human or animal disorders or conditions. Example of such disorders or conditions include, but are not limited to, metabolic disorders such as type 2 diabetes, impaired glucose tolerance, metabolic syndrome (e.g., syndrome X), and insulin resistance induced by trauma (e.g., burns or nitrogen imbalance); adipose tissue disorders (e.g., obesity); muscle and neuromuscular disorders such as muscular dystrophy (including Duchenne muscular dystrophy); amyotrophic lateral sclerosis (ALS); muscle atrophy; organ atrophy; frailty; carpal tunnel syndrome; congestive obstructive pulmonary disease; and sarcopenia, cachexia and other muscle wasting syndromes. Other examples include osteoporosis, especially in the elderly and/or postmenopausal women; glucocorticoid-induced osteoporosis; osteopenia; osteoarthritis; and osteoporosis-related fractures. Yet further examples include low bone mass due to chronic glucocorticoid therapy, premature gonadal failure, androgen suppression, vitamin D deficiency, secondary hyperparathyroidism, nutritional deficiencies, and anorexia nervosa. These disorders and conditions are discussed below under "Exemplary Therapeutic Uses."

The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention 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 invention 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 the term is used.

"About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Typically, exemplary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values.

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

are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

The methods of the invention may include steps of comparing sequences to each other, including an unmodified (wild-type) sequence to one or more mutants (sequence variants). Such comparisons typically comprise alignments of polymer sequences, e.g., using sequence alignment programs and/or algorithms that are well known in the art (for example, BLAST, FASTA and MEGALIGN, to name a few). The skilled artisan can readily appreciate that, in such alignments, where a mutation contains a residue insertion or deletion, the sequence alignment will introduce a "gap" (typically represented by a dash, or "A") in the polymer sequence not containing the inserted or deleted residue.

"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.

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.

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.

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

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

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

may mean values that are within an order of magnitude, preferably ≤ 5 -fold and more preferably ≤ 2-fold of a given value.

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

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

2. ActRIIB Polypeptides

In certain aspects, the invention relates to ActRIIB variant polypeptides (e.g., soluble ActRIIB polypeptides). Optionally, the fragments, functional variants, and modified forms have similar or the same biological activities of their corresponding wild-type ActRIIB polypeptides. For example, an ActRIIB variant of the invention may bind to and inhibit function of an ActRIIB ligand (e.g., activin A, activin AB, activin B, Nodal, GDF8, GDF11 or BMP7). Optionally, an ActRIIB polypeptide modulates growth of tissues such as bone, cartilage, muscle or fat. Examples of ActRIIB polypeptides include human ActRIIB precursor polypeptide (SEQ ID NO: 2), and soluble human ActRIIB polypeptides (e.g., SEQ ID NOs: 1, 5, 6 and 12).

The disclosure identifies functionally active portions and variants of ActRIIB. Applicants have ascertained that an Fc fusion protein having the sequence disclosed by Hilden et al. (Blood. 1994 Apr 15;83(8):2163-70), which has an alanine at the position corresponding to amino acid 64 of SEQ ID NO: 2 (A64), has a relatively low affinity for activin and GDF11. By contrast, the same Fc fusion protein with an arginine at position 64 (R64) has an affinity for activin and GDF-11 in the low nanomolar to high picomolar range. Therefore, a sequence with an R64 is used as the wild-type reference sequence for human ActRIIB in this disclosure.

Attisano et al. (Cell. 1992 Jan 10;68(1):97-108) 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. Data disclosed in WO2008097541 show that an ActRIIB-Fc fusion protein containing amino acids 20-119 of SEQ ID NO:2, "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. 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 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 are not expected to alter ligand binding affinity by large margins. In support of this, mutations of P129 and P130 do not substantially decrease ligand binding. Therefore, an ActRIIB-Fc fusion protein may end as early as amino acid 109 (the final cysteine), however, forms ending at or between 109 and 119 are expected to have reduced ligand binding. Amino acid 119 is poorly conserved and so is readily altered or truncated. Forms ending at 128 or later retain ligand binding activity. Forms ending at or between 119 and 127 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 will retain ligand binding activity. Amino acid 29 represents the initial cysteine. An alanine-to-asparagine mutation at position 24 introduces an N-linked glycosylation sequence without substantially affecting ligand binding. 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, constructs beginning at position 20, 21, 22, 23 and 24 will retain activity, and constructs beginning at positions 25, 26, 27, 28 and 29 are also expected to retain activity. Data are shown in WO2008097541 demonstrates that, surprisingly, a construct beginning at 22, 23, 24 or 25 will have the most activity.

Taken together, an active portion of ActRIIB comprises amino acids 29-109 of SEQ ID NO:2, and constructs may, for example, begin at a residue corresponding to amino acids 20-29 and end at a position corresponding to amino acids 109-134. Other examples include constructs that begin at a position from 20-29 or 21-29 and end at a position from 119-134, 119-133 or 129-134, 129-133. Other examples include constructs that begin at a position

from 20-24 (or 21-24, or 22-25) and end at a position from 109-134 (or 109-133), 119-134 (or 119-133) or 129-134 (or 129-133). Variants within these ranges are also contemplated, particularly those having at least 80%, 85%, 90%, 95% or 99% identity to the corresponding portion of SEQ ID NO:4.

The disclosure includes the results of an analysis of composite ActRIIB structures, shown in Figure 2, demonstrating that the ligand binding pocket is defined 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, although a K74A mutation is well-tolerated, as are R40A, K55A, F82A and mutations at position L79. R40 is a K in Xenopus, indicating that basic amino acids at this position will be tolerated. Q53 is R in bovine ActRIIB and K in Xenopus ActRIIB, and therefore amino acids including R, K, Q, N and H will be tolerated at this position. Thus, a general formula for an active ActRIIB variant protein is one that comprises amino acids 29-109, but optionally beginning at a position ranging from 20-24 or 22-25 and ending at a position ranging from 129-134, and comprising no more than 1, 2, 5, 10 or 15 conservative amino acid changes in the ligand binding pocket, and zero, one or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand binding pocket. Such a protein may retain greater than 80%, 90%, 95% or 99% sequence identity to the sequence of amino acids 29-109 of SEQ ID NO: 2. 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. 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. 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.

ActRIIB is well-conserved across nearly all vertebrates, with large stretches of the extracellular domain conserved completely. See Figure 3. Many of the ligands that bind to ActRIIB are also highly conserverd. Accordingly, comparisons of ActRIIB sequences from various vertebrate organisms provide insights into residues that may be altered. Therefore, an active, human ActRIIB variant may include one or more amino acids at corresponding positions from the sequence of another vertebrate ActRIIB, or may include a residue that is

similar to that in the human or other vertebrate sequence. The following examples illustrate this approach to defining an active ActRIIB variant. L46 is a valine in Xenopus ActRIIB, and so this position may be altered, and optionally may be altered to another hydrophobic residue, such as V, I or F, or a non-polar residue such as A. E52 is a K in Xenopus, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y and probably A. T93 is a K in Xenopus, indicating that a wide structural variation is tolerated at this position, with polar residues favored, such as S, K, R, E, D, H, G, P, G and Y. F108 is a Y in Xenopus, and therefore Y or other hydrophobic group, such as I, V or L should be tolerated. E111 is K in Xenopus, indicating that charged residues will be tolerated at this position, including D, R, K and H, as well as Q and N. R112 is K in Xenopus, indicating that basic residues are tolerated at this position, including R and H. A at position 119 is relatively poorly conserved, and appears as P in rodents and V in Xenopus, thus essentially any amino acid should be tolerated at this position.

Data disclosed in WO2008097541 demonstrate that the addition of a further N-linked glycosylation site (N-X-S/T) does not affect the activity of an ActRIIB-Fc fusion protein, relative to the ActRIIB(R64)-Fc form. Other NX(T/S) sequences are found at 42-44 (NQS) and 65-67 (NSS), although the latter may not be efficiently glycosylated with the R at position 64. N-X-S/T sequences may be generally introduced at positions outside the ligand binding pocket defined in Figure 2. Particularly suitable sites for the introduction of non-endogenous N-X-S/T sequences include amino acids 20-29, 20-24, 22-25, 109-134, 120-134 or 129-134. N-X-S/T sequences may also be introduced into the linker between the ActRIIB sequence and the Fc or other fusion component. Such a site may be introduced with minimal effort by introducing an N in the correct position with respect to a pre-existing S or T, or by introducing an S or T at a position corresponding to a pre-existing N. Thus, desirable alterations that would create an N-linked glycosylation site are: A24N, R64N, S67N (possibly combined with an N65A alteration), E106N, R112N, G120N, E123N, P129N, A132N, R112S and R112T. Any S that is predicted to be glycosylated may be altered to a T without creating an immunogenic site, because of the protection afforded by the glycosylation. Likewise, any T that is predicted to be glycosylated may be altered to an S. Thus the alterations S67T and S44T are contemplated. Likewise, in an A24N variant, an S26T alteration may be used. Accordingly, an ActRIIB variant may include one or more additional, non-endogenous N-linked glycosylation consensus sequences.

Position L79 may be altered to confer altered activin – myostatin (GDF-11) binding properties. L79A or L79P reduces GDF-11 binding to a greater extent than activin binding. L79E or L79D retains GDF-11 binding. Remarkably, the L79E and L79D variants have greatly reduced activin binding. In vivo experiments indicate that these non-activin receptors retain significant ability to increase muscle mass but show decreased effects on other tissues. These data demonstrate the desirability and feasibility for obtaining polypeptides with reduced effects on activin.

The variations described may be combined in various ways. Additionally, the results of mutagenesis program described herein indicate that there are amino acid positions in ActRIIB that are often beneficial to conserve. These include position 64 (basic amino acid), position 80 (acidic or hydrophobic amino acid), position 78 (hydrophobic, and particularly tryptophan), position 37 (acidic, and particularly aspartic or glutamic acid), position 56 (basic amino acid), position 60 (hydrophobic amino acid, particularly phenylalanine or tyrosine). Thus, in each of the variants disclosed herein, the disclosure provides a framework of amino acids that may be conserved. Other positions that may be desirable to conserve are as follows: position 52 (acidic amino acid), position 55 (basic amino acid), position 81 (acidic), 98 (polar or charged, particularly E, D, R or K).

In certain embodiments, isolated fragments of the ActRIIB polypeptides can be obtained by screening polypeptides recombinantly produced from the corresponding fragment of the nucleic acid encoding an ActRIIB polypeptide (e.g., SEQ ID NOs: 3 and 4). In addition, fragments can be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those peptidyl fragments that can function, for example, as antagonists (inhibitors) or agonists (activators) of an ActRIIB protein or an ActRIIB ligand.

In certain embodiments, a functional variant of the ActRIIB polypeptides has an amino acid sequence that is at least 75% identical to an amino acid sequence selected from SEQ ID NOs: 1, 2, and 53. In certain cases, the functional variant has an amino acid sequence at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 1, 2, and 53.

In certain embodiments, the present invention contemplates making functional variants by modifying the structure of an ActRIIB polypeptide for such purposes as

enhancing therapeutic efficacy, or stability (e.g., ex vivo shelf life and resistance to proteolytic degradation in vivo). Modified ActRIIB polypeptides can also be produced, for instance, by amino acid substitution, deletion, or addition. 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 an ActRIIB polypeptide results in a functional homolog can be readily determined by assessing the ability of the variant ActRIIB polypeptide to produce a response in cells in a fashion similar to the wild-type ActRIIB polypeptide, or to bind to one or more ligands, such as activin, GDF11, or GDF8, in a fashion similar to wild type.

In certain specific embodiments, the present invention contemplates making mutations in the extracellular domain (also referred to as ligand-binding domain) of an ActRIIB polypeptide such that the variant (or mutant) ActRIIB polypeptide has altered ligand-binding activities (e.g., binding affinity or binding selectivity). In certain cases, such variant ActRIIB polypeptides have altered (elevated or reduced) binding affinity for a specific ligand. In other cases, the variant ActRIIB polypeptides have altered binding selectivity for their ligands.

In certain embodiments, the present invention contemplates specific mutations of the ActRIIB polypeptides so as to alter the glycosylation of the polypeptide. Exemplary glycosylation sites in ActRIIB polypeptides are illustrated in Figure 6 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 (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 wild-type ActRIIB 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 an ActRIIB polypeptide is by chemical or

enzymatic coupling of glycosides to the ActRIIB 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. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston (1981) CRC Crit. Rev. Biochem., pp. 259-306, incorporated by reference herein. Removal of one or more carbohydrate moieties present on an ActRIIB polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of the ActRIIB 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. Chemical deglycosylation is further described by Hakimuddin et al. (1987) Arch. Biochem. Biophys. 259:52 and by Edge et al. (1981) Anal. Biochem. 118:131. Enzymatic cleavage of carbohydrate moieties on ActRIIB polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. (1987) Meth. Enzymol. 138:350. The sequence of an ActRIIB 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, ActRIIB proteins for use in humans will 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.

This disclosure further contemplates a method of generating variants, particularly sets of combinatorial variants of an ActRIIB polypeptide, including, optionally, truncation variants; pools of combinatorial mutants are especially useful for identifying functional variant sequences. The purpose of screening such combinatorial libraries may be to generate, for example, ActRIIB polypeptide variants which have altered properties, such as altered pharmacokinetics, or altered ligand binding. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, an ActRIIB polypeptide variant may be screened for ability to bind to an ActRIIB polypeptide, to prevent binding of an ActRIIB ligand to an ActRIIB polypeptide.

The activity of an ActRIIB polypeptide or its variants may also be tested in a cell-based or in vivo assay. For example, the effect of an ActRIIB polypeptide variant on the expression of genes involved in bone production in an osteoblast or precursor may be assessed. This may, as needed, be performed in the presence of one or more recombinant ActRIIB ligand protein (e.g., BMP7), and cells may be transfected so as to produce an ActRIIB polypeptide and/or variants thereof, and optionally, an ActRIIB ligand. Likewise, an ActRIIB polypeptide may be administered to a mouse or other animal, and one or more bone properties, such as density or volume may be assessed. The healing rate for bone fractures may also be evaluated. Similarly, the activity of an ActRIIB polypeptide or its variants may be tested in muscle cells, adipocytes, and neuronal cells for any effect on growth of these cells, for example, by the assays as described below. Such assays are well known and routine in the art. A SMAD-responsive reporter gene may be used in such cell lines to monitor effects on downstream signaling.

Combinatorially-derived variants can be generated which have a selective potency relative to a naturally occurring ActRIIB polypeptide. Such variant proteins, 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 a wild-type ActRIIB polypeptide. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other processes which result in destruction of, or otherwise inactivation of a native ActRIIB polypeptide. Such variants, and the genes which encode them, can be utilized to alter ActRIIB polypeptide levels by modulating the half-life of the ActRIIB polypeptides. 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 ActRIIB polypeptide levels within the cell.

In certain embodiments, the ActRIIB polypeptides of the invention may further comprise post-translational modifications in addition to any that are naturally present in the ActRIIB polypeptides. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, the modified ActRIIB polypeptides may contain non-amino acid elements, such as polyethylene glycols, lipids, poly- or mono-saccharide, and phosphates. Effects of such non-amino acid elements on the functionality of a ActRIIB polypeptide may be tested as described herein for other ActRIIB polypeptide variants. When an ActRIIB polypeptide is produced in cells by cleaving a nascent form of the ActRIIB polypeptide, post-translational processing may also

be important for correct folding and/or function of the protein. Different cells (such as 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 ActRIIB polypeptides.

In certain aspects, functional variants or modified forms of the ActRIIB polypeptides include fusion proteins having at least a portion of the ActRIIB polypeptides and one or more fusion domains. 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 (e.g., an Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. 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 ActRIIB 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 preferred embodiments, an ActRIIB polypeptide is fused with a domain that stabilizes the ActRIIB polypeptide in vivo (a "stabilizer" domain). By "stabilizing" is meant anything that increases serum half life, regardless of whether this is because of decreased destruction, decreased clearance by the kidney, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin are known to confer desirable pharmacokinetic properties on a wide range of proteins. Likewise, fusions to human serum albumin can confer desirable properties. Other types of fusion domains that may be selected include multimerizing (e.g., dimerizing, tetramerizing) domains and

functional domains (that confer an additional biological function, such as further stimulation of muscle growth).

In certain aspects the polypeptides disclosed herein may form homomeric variant ActRIIB polypeptides, meaning that each fusion polypeptide chain in the protein complex comprises the same ActRIIB variant as any other such chain in the complex. In certain aspects, the polypeptides disclosed herein may form heteromultimers comprising at least one variant ActRIIB polypeptide associated, covalently or non-covalently, with at least one unmodified ActRIIB polypeptide or at least one variant ActRIIB polypeptide different from the first ActRIIB variant. In certain aspects, the polypeptides disclosed herein may form heteromultimers comprising at least one variant ActRIIB polypeptide associated, covalently or non-covalently, with 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. In certain aspects, the polypeptides disclosed herein may form heteromultimers comprising at least one variant ActRIIB polypeptide associated, covalently or non-covalently, with at least one TGF-beta superfamily type II serine/threonine kinase receptor polypeptide (*e.g.*, ActRIIA, TGFBRII, BMPRII, and MISRII), including fragments and variants thereof. Preferably, heteromeric polypeptides disclosed herein form heterodimers, although higher order heteromultimers are also included such as, but not limited to, heterotrimers, heterotetramers, and further oligomeric structures. In some embodiments, variant ActRIIB 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. Variant ActRIIB 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.*, variant ActRIIB polypeptide) and a second polypeptide (*e.g.*, an unmodified ActRIIB polypeptide or a variant ActRIIB polypeptide different from that present in the first polypeptide) to promote heteromultimer formation (*e.g.*, heterodimer formation), and optionally hinders or otherwise disfavors homomultimer formation (*e.g.*, homodimer formation), thereby increasing the yield of desired heteromultimer (see, e.g., Figure 1).

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

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

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

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

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

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

In certain embodiments, the disclosure relates to a heteromultimer comprising at least one ALK6-Fc fusion protein and at least one ActRIIB-Fc fusion protein. In some

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

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

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

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

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

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

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK1-Fc fusion protein. In some embodiments, the ALK1-Fc fusion protein comprises an ALK1 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids of 22-34 (e.g., amino acid residues 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, and 34) SEQ ID NO: 54, 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: 54. In some embodiments, the ALK1-Fc fusion protein comprises an ALK1 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22-118 of SEQ ID NO: 54. In some embodiments, the ALK1-Fc fusion protein comprises an ALK1 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-95 of SEQ ID NO: 54. In some embodiments, the ALK1-Fc fusion protein comprises an ALK1 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63.

A representative ALK1-Fc fusion polypeptide (SEQ ID NO: 60) 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: 60)
```

The leader sequence and linker sequence are underlined.

The mature ALK1-Fc fusion protein sequence (SEQ ID NO: 61) 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: 61)
```

In some embodiments, the ALK1-Fc fusion polypeptide (SEQ ID NO: 56) 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: 56)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with certain Fc fusion polypeptides disclosed herein, 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: 56 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: 258):

```
  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: 258)
```

The mature ALK1-Fc fusion protein sequence (SEQ ID NO: 57) 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: 57)
```

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK2 polypeptide. As used herein, the term "ALK2" refers to a family of activin receptor-like kinase-2 proteins from any species and variants derived from such ALK2 proteins by mutagenesis or other modification. Reference to ALK2 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK2 family are generally

transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

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

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

1 MVDGVMILPV LIMIALPSPS **MEDEKPKVNP KLYMCVCEGL SCGNEDHCEG**
51 QQCFSSLSIN DGFHVYQKGC FQVYEQGKMT CKTPPSPGQA VECCQGDWCN
101 RNITAQLPTK GKSFPGTQNF 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: 64)

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

A processed extracellular ALK2 polypeptide sequence is as follows:

MEDEKPKVNPKLYMCVCEGLSCGNEDHCEGQQCFSSLSINDGFHVYQKGCFQVYEQGKMTCK
TPPSPGQAVECCQGDWCNRNITAQLPTKGKSFPGTQNFHLE (SEQ ID NO: 65)

A nucleic acid sequence encoding human ALK2 precursor protein is shown in SEQ ID NO: 217, corresponding to nucleotides 431-1957 of Genbank Reference Sequence NM_001105.4. A nucleic acid sequence encoding the extracellular ALK2 polypeptide is as in SEQ ID NO: 218.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK2 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK2 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK2). In other preferred embodiments, ALK2

polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 64 or 65. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one ALK2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 64 or 65.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK2-Fc fusion protein. In some embodiments, the ALK2-Fc fusion protein comprises an ALK2 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 21-35 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, and 35) SEQ ID NO: 64, and ends at any one of amino acids 99-123 (e.g., amino acid residues 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, and 123) of SEQ ID NO: 64. In some embodiments, the ALK2-Fc fusion protein comprises an ALK2 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 35-99 of SEQ ID NO: 64. In some embodiments, the ALK2-Fc fusion protein comprises an ALK2 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-123 of SEQ ID NO: 64. In some embodiments, the ALK2-Fc fusion protein comprises an ALK2 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID Nos: 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73.

In some embodiments, the ALK2-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 66):

```
  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: 66)
```

The signal sequence and linker sequence are underlined. To promote formation of the a heterodimer with certain other Fc fusions disclosed herein, 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: 66 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: 244):

```
   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: 244)
```

The mature ALK2-Fc fusion protein sequence (SEQ ID NO: 67) 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: 67)
```

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.

In some embodiments, the ALK2-Fc fusion polypeptide (SEQ ID NO: 70) 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: 70)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with certain Fc fusion polypeptides disclosed herein, 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: 70 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK2-Fc fusion protein sequence (SEQ ID NO: 71) 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: 71)

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

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

A representative 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: 64)

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

A processed extracellular ALK2 polypeptide sequence is as follows:

MEDEKPKVNPKLYMCVCEGLSCGNEDHCEGQQCFSSLSINDGFHVYQKGCFQVYEQGKMTCK
TPPSPGQAVECCQGDWCNRNITAQLPTKGKSFPGTQNFHLE (SEQ ID NO: 65)

A nucleic acid sequence encoding human ALK2 precursor protein is shown in SEQ ID NO: 217, corresponding to nucleotides 431-1957 of Genbank Reference Sequence NM_001105.4. A nucleic acid sequence encoding the extracellular ALK2 polypeptide is as in SEQ ID NO: 218.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK2 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK2 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK2 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK2). In other preferred embodiments, ALK2 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 64 or 65. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one ALK2 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 64 or 65.

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

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

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

```
  1 MPQLYIYIRL LGAYLFIISR VQGQNLDSML HGTGMKSDSD QKKSENGVTL APEDTLPFLK
 61 CYCSGHCPDD AINNTCITNG HCFAIIEEDD QGETTLASGC MKYEGSDFQC KDSPKAQLRR
```

121 TIECCRTNLC NQYLQPTLPP VVIGPFFDGS IRWLVLLISM AVCIIAMIIF SSCFCYKHYC
181 KSISSRRRYN RDLEQDEAFI PVGESLKDLI DQSQSSGSGS GLPLLVQRTI AKQIQMVRQV
241 GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS WFRETEIYQT VLMRHENILG FIAADIKGTG
301 SWTQLYLITD YHENGSLYDF LKCATLDTRA LLKLAYSAAC GLCHLHTEIY GTQGKPAIAH
361 RDLKSKNILI KKNGSCCIAD LGLAVKFNSD TNEVDVPLNT RVGTKRYMAP EVLDESLNKN
421 HFQPYIMADI YSFGLIIWEM ARRCITGGIV EEYQLPYYNM VPSDPSYEDM REVVCVKRLR
481 PIVSNRWNSD ECLRAVLKLM SECWAHNPAS RLTALRIKKT LAKMVESQDV KI
(SEQ ID NO: 74)

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

A processed extracellular ALK3 polypeptide sequence is as follows:

1 QNLDSMLHGT GMKSDSDQKK SENGVTLAPE DTLPFLKCYC SGHCPDDAIN NTCITNGHCF
61 AIIEEDDQGE TTLASGCMKY EGSDFQCKDS PKAQLRRTIE CCRTNLCNQY LQPTLPPVVI
121 GPFFDGSIR (SEQ ID NO: 75)

A nucleic acid sequence encoding human ALK3 precursor protein is shown in SEQ ID NO: 219, corresponding to nucleotides 549-2144 of Genbank Reference Sequence NM_004329.2. The signal sequence is underlined and the extracellular domain is indicated in **bold** font. A nucleic acid sequence encoding the extracelluar human ALK3 polypeptide is shown in SEQ ID NO: 220.

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: 74 and ends at any amino acid position 140-152 of SEQ ID NO: 74 (*i.e.*, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, or 152). See U.S. Patent 8,338,377, the teachings of which are incorporated herein by reference in their entirety.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK3 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK3 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK3 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK3). In other preferred embodiments, ALK3 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the

disclosure comprise at least one ALK3 polypeptide that comprises an amino acid beginning at any amino acid position 25-31 (*i.e.*, position 25, 26, 27, 28, 29, 30, or 31) of SEQ ID NO: 74 and ending at any amino acid position 140-153 of SEQ ID NO: 74 (*i.e.*, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, or 152) of SEQ ID NO: 74. In some embodiments, heteromultimer complexes of the disclosure comprise at least one ALK3 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 74, 75, 76, 77, 80, or 81. 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: 74, 75, 76, 77, 80, or 81.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK3-Fc fusion protein. In some embodiments, the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 24-61 (e.g., amino acid residues 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, and 61) SEQ ID NO: 74, and ends at any one of amino acids 130-152 (e.g., amino acid residues 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, and 152) of SEQ ID NO: 74. In some embodiments, the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 61-130 of SEQ ID NO: 74. In some embodiments, the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-152 of SEQ ID NO: 74. In some embodiments, the ALK3-Fc fusion protein comprises an ALK3 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 74, 75, 76, 77, 78, 79, 80, 81, 82, and 83.

In some embodiments, the ALK3-Fc fusion protein employs the TPA leader and is as follows:

```
  1 MDAMKRGLCC VLLLCGAVFV SPGAQNLDSM LHGTGMKSDS DQKKSENGVT
 51 LAPEDTLPFL KCYCSGHCPD DAINNTCITN GHCFAIIEED DQGETTLASG
```

```
101 CMKYEGSDFQ CKDSPKAQLR RTIECCRTNL CNQYLQPTLP PVVIGPFFDG
151 SIRTGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
201 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
251 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
301 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF LYSDLTVDKS
351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G  (SEQ ID NO: 76)
```

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: 76 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: 245).

```
   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: 245)

The mature ALK3-Fc fusion protein sequence is as follows (SEQ ID NO: 77) 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: 77)
```

The complementary form of ALK3-Fc fusion polypeptide (SEQ ID NO: 80) 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: 80)
```

The leader sequence and linker are underlined. To guide heterodimer formation with certain Fc fusion polypeptides disclosed herein, 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: 80 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK3-Fc fusion protein sequence (SEQ ID NO: 81) 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: 81)

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

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

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

1 MAESAGASSF FPLVVLLLAG 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: 84)

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

A processed extracellular human ALK4 polypeptide sequence is as follows:

SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGKPFYCL
SSEDLRNTHCCYTDYCNRIDLRVPSGHLKEPEHPSMWGPVE (SEQ ID NO: 86)

A nucleic acid sequence encoding an ALK4 precursor protein is shown in SEQ ID NO: 221), corresponding to nucleotides 78-1592 of Genbank Reference Sequence NM_004302.4. A nucleic acid sequence encoding the extracellular ALK4 polypeptide is shown in SEQ ID NO: 222.

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: 85)

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

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

SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGKPFYCL SSEDLRNTHCCYTDYCNRIDLRVPSGHLKEPEHPSMWGPVE (SEQ ID NO: 87)

A nucleic acid sequence encoding an ALK4 precursor protein (isoform C) is shown in SEQ ID NO: 223, corresponding to nucleotides 78-1715 of Genbank Reference Sequence NM_020328.3. A nucleic acid sequence encoding the extracelluar ALK4 polypeptide (isoform C) is shown in SEQ ID NO: 224.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK4 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK4 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK4). In other preferred embodiments, ALK4 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK4 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 84, 86, 85, 87, 88, 89, 92, or 93. In some embodiments, heteromultimers of the disclosure consist or consist essentially of at least one ALK4 polypeptide that is at least 70%, 75%,

80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 84, 86, 85, 87, 88, 89, 92, or 93.

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

In certain embodiments, the polypeptide comprises an ALK4-Fc fusion polypeptide (SEQ ID NO: 88) 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: 88)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with certain Fc fusion polypeptides of the disclosure, 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: 88 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: 243):

```
   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:243)
```

The mature ALK4-Fc fusion protein sequence (SEQ ID NO: 89) 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: 89)
```

In some embodiments, the ALK4-Fc fusion polypeptide (or any Fc fusion polypeptide disclosed herein) employs the tissue plasminogen activator (TPA) leader: MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 246).

In some embodiments, the ALK4-Fc fusion polypeptide (SEQ ID NO: 92) 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: 92)
```

The leader sequence and the linker are underlined. To guide heterodimer formation with certain Fc fusion polypeptides disclosed herein, 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: 92 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: 93)
```

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

In some embodiments, the ALK4-Fc fusion polypeptide (SEQ ID NO: 247) is as follows and may optionally be provided with lysine removed from the C-terminus.

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
 51  GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101  YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151  LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201  REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251  QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESRGQPENNY
301  KTTPPVLDSR GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351  SLSPGK      (SEQ ID NO: 247)
```

The leader sequence and the linker are underlined. To guide heterodimer formation with certain Fc fusion polypeptides disclosed herein, four amino acid substitutions (replacing a tyrosine with a cysteine, a threonine with a serine, a leucine with an alanine, and a tyrosine with a valine) can be introduced into the Fc domain of the ALK4 fusion polypeptide as indicated by double underline above. To facilitate purification of the ALK4-Fc:ActRIIB-Fc heterodimer, two amino acid substitutions (replacing an asparagine with an arginine and an aspartate with an arginine) can also be introduced into the Fc domain of the ALK4-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 247 may optionally be provided with lysine removed from the C-terminus.

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 248):

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

```
 651  CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA
 701  ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
 751  CAGCCCCGAG AACCACAGGT GTGCACCCTG CCCCCATCCC GGGAGGAGAT
 801  GACCAAGAAC CAGGTCAGCC TGTCCTGCGC CGTCAAAGGC TTCTATCCCA
 851  GCGACATCGC CGTGGAGTGG GAGAGCCGCG GGCAGCCGGA GAACAACTAC
 901  AAGACCACGC CTCCCGTGCT GGACTCCCGC GGCTCCTTCT TCCTCGTGAG
 951  CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001  GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
1051  TCCCTGTCTC CGGGTAAA      (SEQ ID NO: 248)
```

The mature ALK4-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 249) and may optionally be provided with lysine removed from the C-terminus.

```
   1  SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
  51  ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
 101  PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 151  VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 201  GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
 251  SCAVKGFYPS DIAVEWESRG QPENNYKTTP PVLDSRGSFF LVSKLTVDKS
 301  RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK   (SEQ ID NO: 249)
```

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 250):

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

651 CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
701 GCACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
751 TCCTGCGCCG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA
801 GAGCCGCGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG
851 ACTCCCGCGG CTCCTTCTTC CTCGTGAGCA AGCTCACCGT GGACAAGAGC
901 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
951 GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
(SEQ ID NO: 250)

In certain embodiments, the ALK4-Fc fusion polypeptide is SEQ ID NO: 92 (shown above), which contains four amino acid substitutions to guide heterodimer formation certain Fc fusion polypeptides disclosed herein, and may optionally be provided with lysine removed from the C-terminus.

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 251):

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

```
 951  CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001  GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
1051  TCCCTGTCTC CGGGTAAA      (SEQ ID NO: 251)
```

The mature ALK4-Fc fusion polypeptide sequence is SEQ ID NO: 93 (shown above) and may optionally be provided with lysine removed from the C-terminus.

This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 252):

```
   1  TCCGGGCCCC GGGGGGTCCA GGCTCTGCTG TGTGCGTGCA CCAGCTGCCT
  51  CCAGGCCAAC TACACGTGTG AGACAGATGG GGCCTGCATG GTTTCCATTT
 101  TCAATCTGGA TGGGATGGAG CACCATGTGC GCACCTGCAT CCCCAAAGTG
 151  GAGCTGGTCC CTGCCGGGAA GCCCTTCTAC TGCCTGAGCT CGGAGGACCT
 201  GCGCAACACC CACTGCTGCT ACACTGACTA CTGCAACAGG ATCGACTTGA
 251  GGGTGCCCAG TGGTCACCTC AAGGAGCCTG AGCACCCGTC CATGTGGGGC
 301  CCGGTGGAGA CCGGTGGTGG AACTCACACA TGCCCACCGT GCCCAGCACC
 351  TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG
 401  ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
 451  GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT
 501  GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA
 551  CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT
 601  GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT
 651  CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT
 701  GCACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG
 751  TCCTGCGCCG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA
 801  GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG
 851  ACTCCGACGG CTCCTTCTTC CTCGTGAGCA AGCTCACCGT GGACAAGAGC
 901  AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
 951  GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
      (SEQ ID NO: 252)
```

Purification of various ALK4-Fc:ActRIIB-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography and epitope-based affinity chromatography (e.g., with an antibody or functionally equivalent ligand directed against an

epitope on ALK4 or ActRIIB), and multimodal chromatography (e.g., with resin containing both electrostatic and hydrophobic ligands). The purification could be completed with viral filtration and buffer exchange.

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

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

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

```
  1 MEAAVAAPRP RLLLLVLAAA AAAAALLPG ATALQCFCHL CTKDNFTCVT DGLCFVSVTE
 61 TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE LPTTVKSSPG
121 LGPVELAAVI AGPVCFVCIS LMLMVYICHN RTVIHHRVPN EEDPSLDRPF ISEGTTLKDL
181 IYDMTTSGSG SGLPLLVQRT IARTIVLQES IGKGRFGEVW RGKWRGEEVA VKIFSSREER
241 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS DYHEHGSLFD YLNRYTVTVE
301 GMIKLALSTA SGLAHLHMEI VGTQGKPAIA HRDLKSKNIL VKKNGTCCIA DLGLAVRHDS
361 ATDTIDIAPN HRVGTKRYMA PEVLDDSINM KHFESFKRAD IYAMGLVFWE IARRCSIGGI
421 HEDYQLPYYD LVPSDPSVEE MRKVVCEQKL RPNIPNRWQS CEALRVMAKI MRECWYANGA
481 ARLTALRIKK TLSQLSQQEG IKM (SEQ ID NO: 96)
```

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

A processed extracellular ALK5 polypeptide sequence is as follows:

```
AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVCAPSSKTGSVTTTY
CCNQDHCNKIELPTTVKSSPGLGPVEL (SEQ ID NO: 98)
```

A nucleic acid sequence encoding the ALK5 precursor protein is shown in SEQ ID NO: 225, corresponding to nucleotides 77-1585 of Genbank Reference Sequence

NM_004612.2. A nucleic acid sequence encoding an extracellular human ALK5 polypeptide is shown in SEQ ID NO: 226.

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

```
  1 MEAAVAAPRP RLLLLVLAAA AAAAALLPG ATALQCFCHL CTKDNFTCVT DGLCFVSVTE
 61 TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE LPTTGPFSVK
121 SSPGLGPVEL AAVIAGPVCF VCISLMLMVY ICHNRTVIHH RVPNEEDPSL DRPFISEGTT
181 LKDLIYDMTT SGSGSGLPLL VQRTIARTIV LQESIGKGRF GEVWRGKWRG EEVAVKIFSS
241 REERSWFREA EIYQTVMLRH ENILGFIAAD NKDNGTWTQL WLVSDYHEHG SLFDYLNRYT
301 VTVEGMIKLA LSTASGLAHL HMEIVGTQGK PAIAHRDLKS KNILVKKNGT CCIADLGLAV
361 RHDSATDTID IAPNHRVGTK RYMAPEVLDD SINMKHFESF KRADIYAMGL VFWEIARRCS
421 IGGIHEDYQL PYYDLVPSDP SVEEMRKVVC EQKLRPNIPN RWQSCEALRV MAKIMRECWY
481 ANGAARLTAL RIKKTLSQLS QQEGIKM (SEQ ID NO: 97)
```

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

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

```
AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVC
APSSKTGSVTTTYCCNQDHCNKIELPTTGPFSVKSSPGLGPVEL (SEQ ID NO: 99)
```

A nucleic acid sequence encoding human ALK5 precursor protein (isoform 2) is shown in SEQ ID NO: 227, corresponding to nucleotides 77-1597 of Genbank Reference Sequence XM_005252150.1. A nucleic acid sequence encoding a processed extracellular ALK5 polypeptide is shown in SEQ ID NO: 228.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK5 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK5 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK5). In other preferred embodiments, ALK5 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96, 98, 97, or 99. In some embodiments, heteromultimer complexes of the disclosure consist

or consist essentially of at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96, 98, 97, or 99.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK5-Fc fusion protein. In some embodiments, the ALK5-Fc fusion protein comprises an ALK5 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 25-36 (e.g., amino acid residues 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36) SEQ ID NO: 96 or 97, and ends at any one of amino acids 106-126 (e.g., amino acid residues 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, and 126) of SEQ ID NO: 96 or 97. In some embodiments, the ALK5-Fc fusion protein comprises an ALK5 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 36-106 of SEQ ID NOs: 96 or 97. In some embodiments, the ALK5-Fc fusion protein comprises an ALK5 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-126 of SEQ ID NOs: 96 or 97. In some embodiments, the ALK5-Fc fusion protein comprises an ALK5 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 96, 98, 97, 99, 100, 101, 102, 103, 104, 105, 106, and 107.

The complementary ALK5-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 100):

```
  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: 100)
```

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: 100 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: 253):

```
   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: 253)
```

The mature ALK5-Fc fusion protein sequence (SEQ ID NO: 101) 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: 101)
```

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.

In some embodiments, the ALK5-Fc fusion polypeptide (SEQ ID NO: 104) 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: 104)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with certain Fc fusion polypeptides disclosed herein, 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: 104 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK5-Fc fusion protein sequence (SEQ ID NO: 105) 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: 105)

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

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

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

1 MLLRSAGKLN 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: 108)

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:

KKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMIEEDDSGLPVVTSGCLGLEGSDFQCRDTPIPH
QRRSIECCTERNECNKDLHPTLPPLKNRDFVDGPIHHR (SEQ ID NO: 109)

A nucleic acid sequence encoding the ALK6 precursor protein is shown in SEQ ID NO: 229, corresponding to nucleotides 275-1780 of Genbank Reference Sequence

NM_001203.2. A nucleic acid sequence encoding processed extracellular ALK6 polypeptide is shown in SEQ ID NO: 230.

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: 110)

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

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

NFLDNMLLRSAGKLNVGTKKEDGESTAPTPRPKVLRCKCHHHCPEDSVNNICSTDGYCFTMIEEDDSGLPVVTSG CLGLEGSDFQCRDTPIPHQRRSIECCTERNECNKDLHPTLPPLKNRDFVDGPIHHR (SEQ ID NO: 111)

A nucleic acid sequence encoding human ALK6 precursor protein (isoform 2) is shown in SEQ ID NO: 231, corresponding to nucleotides 22-1617 of Genbank Reference Sequence NM_001256793.1. A nucleic acid sequence encoding a processed extracellular ALK6 polypeptide is shown in SEQ ID NO: 232.

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

108, 109, 110, or 111. In some embodiments, heteromultimers of the disclosure consist or consist essentially of at least one ALK6 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 108, 109, 110, or 111.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK6-Fc fusion protein. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 14-32 (e.g., amino acid residues 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, and 32) SEQ ID NO: 108, and ends at any one of amino acids 102-126 (e.g., amino acid residues 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, and 126) of SEQ ID NO: 108. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 32-102 of SEQ ID NO: 108. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 14-126 of SEQ ID NO: 108. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, and 119.

In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 26-62 (e.g., amino acid residues 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62) SEQ ID NO: 110, and ends at any one of amino acids 132-156 (e.g., amino acid residues 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, and 156) of SEQ ID NO: 110. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 62-132 of SEQ ID NO: 110. In some embodiments, the ALK6-Fc fusion protein comprises an ALK6 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 26-156 of SEQ ID NO: 110.

The complementary ALK6-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 112):

```
  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: 112)
```

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: 112 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: 254):

```
  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: 254)

The mature ALK6-Fc fusion protein sequence (SEQ ID NO: 113) 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: 113)

In another approach to promoting 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 complementary form of ALK6-Fc fusion polypeptide (SEQ ID NO: 116) 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: 116)

The leader sequence and linker sequence are underlined. To guide heterodimer formation with certain Fc fusion polypeptides disclosed herein, 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: 116 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK6-Fc fusion protein sequence (SEQ ID NO: 117) 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: 117)

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

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

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

```
  1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI
 61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PMELAIIITV
121 PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL IYDVTASGSG
181 SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ
241 TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA
301 SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN
361 PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD
421 MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK
481 TISQLCVKED CKA (SEQ ID NO: 120)
```

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

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

ELSPGLKCVCLLCDSSNFTCQTEGACWASVMLTNGKEQVIKSCVSLPELNAQVFCHSSNNVTKTECCFTDFCNNI TLHLPTASPNAPKLGPME (SEQ ID NO: 123)

A nucleic acid sequence encoding human ALK7 isoform 1 precursor protein is shown below in SEQ ID NO: 233, corresponding to nucleotides 244-1722 of Genbank Reference Sequence NM_145259.2. A nucleic acid sequence encoding the processed extracellular ALK7 polypeptide (isoform 1) is show in in SEQ ID NO: 234.

An amino acid sequence of an alternative isoform of human ALK7, isoform 2 (NCBI Ref Seq NP_001104501.1), is shown in its processed form as follows (SEQ ID NO: 124), where the extracellular domain is indicated in **bold** font.

```
  1 MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG
 61 PMELAIIITV PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL
121 IYDVTASGSG SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER
181 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA
241 GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS
301 ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI
361 VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA
421 ARLTALRIKK TISQLCVKED CKA  (SEQ ID NO: 124)
```

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

MLTNGKEQVIKSCVSLPELNAQVFCHSSNNVTKTECCFTDFCNNITLHLPTASPNAPK LGPME (SEQ ID NO: 125).

A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 2) is shown below in SEQ ID NO: 235, corresponding to nucleotides 279-1607 of NCBI Reference Sequence NM_001111031.1.

A nucleic acid sequence encoding an extracellular ALK7 polypeptide (isoform 2) is shown in SEQ ID NO: 236.

An amino acid sequence of an alternative human ALK7 precursor protein, isoform 3 (NCBI Ref Seq NP_001104502.1), is shown as follows (SEQ ID NO: 121), 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: 121)
```

The amino acid sequence of a processed ALK7 polypeptide (isoform 3) is as follows (SEQ ID NO: 126). 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: 126 are predicted as described below.

```
  1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN
 61 VTKTECCFTD FCNNITLHLP TGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA
121 VKIFSSRDER SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD
181 YLNRNIVTVA GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA
241 DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE
301 IARRCSVGGI VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI
361 MRECWYANGA ARLTALRIKK TISQLCVKED CKA  (SEQ ID NO: 126)
```

A nucleic acid sequence encoding an unprocessed ALK7 polypeptide precursor protein (isoform 3) is shown in SEQ ID NO: 237, corresponding to nucleotides 244-1482 of NCBI Reference Sequence NM_001111032.1. A nucleic acid sequence encoding a processed ALK7 polypeptide (isoform 3) is shown in SEQ ID NO: 238.

An amino acid sequence of an alternative human ALK7 precursor protein, isoform 4 (NCBI Ref Seq NP_001104503.1), is shown as follows (SEQ ID NO: 122), 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: 122)
```

An amino acid sequence of a processed ALK7 polypeptide (isoform 4) is as follows (SEQ ID NO: 127). 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: 127 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: 127)
```

A nucleic acid sequence encoding the unprocessed ALK7 polypeptide precursor protein (isoform 4) is shown in SEQ ID NO: 239, corresponding to nucleotides 244-1244 of NCBI Reference Sequence NM_001111033.1. A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 4) is shown in SEQ ID NO: 240.

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: 128).

```
  1 LKCVCLLCDS SNFTCQTEGA CWASVMLTNG KEQVIKSCVS LPELNAQVFC HSSNNVTKTE
 61 CCFTDFCNNI TLHLPTASPN APKLGPME          (SEQ ID NO: 128)
```

Active variants of processed ALK7 isoform 1 are predicted in which SEQ ID NO: 123 is truncated by 1, 2, 3, 4, 5, 6, or 7 amino acids at the N-terminus and SEQ ID NO: 128 is truncated by 1 or 2 amino acids at the N-terminus. Consistent with SEQ ID NO: 128, it is further expected that leucine is the N-terminal amino acid in the processed forms of human ALK7 isoform 3 (SEQ ID NO: 126) and human ALK7 isoform 4 (SEQ ID NO: 127).

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK7 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK7 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK7). In other preferred embodiments, ALK7 polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK7 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 120, 123, 129, 130, 124, 125, 121, 126, 122, 127, 128, 133, or 134. In some embodiments, heteromultimers of the disclosure consist or consist essentially of at least one ALK7 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 120, 123, 129, 130, 124, 125, 121, 126, 122, 127, 128, 133, or 134.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ALK7-Fc fusion protein. In some embodiments, the ALK7-Fc fusion protein comprises an ALK7 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 21-28 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, and 28) SEQ ID NO: 120, 121, or 122, and ends at any one of amino acids 92-113 (e.g., amino acid residues 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, and 113) of SEQ ID NO: 120, 121, or 122. In some embodiments, the ALK7-Fc fusion protein comprises an ALK7 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NOs: 120, 121, or 122. In some embodiments, the ALK7-Fc fusion protein comprises an ALK7 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-113 of SEQ ID NOs: 120, 121, or 122. In some embodiments, the ALK7-Fc fusion protein comprises an ALK7 domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any

one of SEQ ID Nos: 120, 123, 124, 125, 121, 126, 122, 127, 128, 129, 130, 131, 132, 133, 134, 135, and 136.

In some embodiments, the ALK7-Fc fusion protein employs the TPA leader and is as follows (SEQ ID NO: 129):

```
  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: 129)
```

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: 129 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: 255):

```
   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: 255)
```

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 130) 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: 130)
```

The complementary form of ALK7-Fc fusion polypeptide (SEQ ID NO: 133) 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: 133)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with certain Fc fusion polypeptides disclosed herein, 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: 133 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 134) 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: 134)
```

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

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

The human ActRIIA precursor protein sequence is as follows:

```
  1 MGAAAKLAFA VFLISCSSGA ILGRSETQEC LFFNANWEKD RTNQTGVEPC
 51 YGDKDKRRHC FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKKDSPEV
101 YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPPYYNIL LYSLVPLMLI
151 AGIVICAFWV YRHHKMAYPP VLVPTQDPGP PPPSPLLGLK PLQLLEVKAR
201 GRFGCVWKAQ LLNEYVAVKI FPIQDKQSWQ NEYEVYSLPG MKHENILQFI
```

251 GAEKRGTSVD VDLWLITAFH EKGSLSDFLK ANVVSWNELC HIAETMARGL
301 AYLHEDIPGL KDGHKPAISH RDIKSKNVLL KNNLTACIAD FGLALKFEAG
351 KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR
401 CTAADGPVDE YMLPFEEEIG QHPSLEDMQE VVVHKKKRPV LRDYWQKHAG
451 MAMLCETIEE CWDHDAEARL SAGCVGERIT QMQRLTNIIT TEDIVTVVTM
501 VTNVDFPPKE SSL (SEQ ID NO: 137)

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: 138)

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: 139)

A nucleic acid sequence encoding the human ActRIIA precursor protein is shown in SEQ ID NO: 241, corresponding to nucleotides 159-1700 of Genbank Reference Sequence NM_001616.4. A nucleic acid sequence encoding a processed extracellular ActRIIA polypeptide is as shown in SEQ ID NO: 242.

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: 137. 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: 137. 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: 137 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: 137.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ActRIIA polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ActRIIA polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ActRIIA). In other preferred embodiments, ActRIIA polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIA polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 137, 138, 139, 140, 141, 144, or 145. In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIA polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 137, 138, 139, 140, 141, 144, or 145.

In certain aspects, the disclosure relates to a heteromultimer that comprises an ActRIIA-Fc fusion protein. In some embodiments, the ActRIIA-Fc fusion protein comprises an ActRIIA domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 21-30 (e.g., amino acid residues 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) SEQ ID NO: 137, and ends at any one of amino acids 110-135 (e.g., 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134 or 135) of SEQ ID NO: 137. In some embodiments, the ActRIIA-Fc fusion protein comprises an ActRIIA domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 137. In some embodiments, the ActRIIA-Fc fusion protein comprises an ActRIIA domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-135 of SEQ ID NO: 137. In some embodiments, the ActRIIA-Fc fusion protein comprises an ActRIIA domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, and 147.

The ActRIIA-Fc polypeptide sequence (SEQ ID NO: 140) 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: 140)
```

The leader sequence and linker sequence 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 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: 140 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: 256):

```
  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: 256)
```

The mature ActRIIA-Fc fusion polypeptide (SEQ ID NO: 141) 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: 141)
```

The ActRIIA-Fc polypeptide sequence (SEQ ID NO: 144) 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: 144)
```

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: 144 may optionally be provided with the lysine removed from the C-terminus.

The mature ActRIIA-Fc fusion polypeptide (SEQ ID NO: 145) 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: 145)
```

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

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

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

```
  1 MTSSLQRPWR VPWLPWTILL VSTAAASQNQ ERLCAFKDPY QQDLGIGESR
 51 ISHENGTILC SKGSTCYGLW EKSKGDINLV KQGCWSHIGD PQECHYEECV
101 VTTTPPSIQN GTYRFCCCST DLCNVNFTEN FPPPDTTPLS PPHSFNRDET
151 IIIALASVSV LAVLIVALCF GYRMLTGDRK QGLHSMNMME AAASEPSLDL
201 DNLKLLELIG RGRYGAVYKG SLDERPVAVK VFSFANRQNF INEKNIYRVP
251 LMEHDNIARF IVGDERVTAD GRMEYLLVME YYPNGSLCKY LSLHTSDWVS
301 SCRLAHSVTR GLAYLHTELP RGDHYKPAIS HRDLNSRNVL VKNDGTCVIS
```

351 DFGLSMRLTG NRLVRPGEED NAAISEVGTI RYMAPEVLEG AVNLRDCESA
401 LKQVDMYALG LIYWEIFMRC TDLFPGESVP EYQMAFQTEV GNHPTFEDMQ
451 VLVSREKQRP KFPEAWKENS LAVRSLKETI EDCWDQDAEA RLTAQCAEER
501 MAELMMIWER NKSVSPTVNP MSTAMQNERN LSHNRRVPKI GPYPDYSSSS
551 YIEDSIHHTD SIVKNISSEH SMSSTPLTIG EKNRNSINYE RQQAQARIPS
601 PETSVTSLST NTTTTNTTGL TPSTGMTTIS EMPYPDETNL HTTNVAQSIG
651 PTPVCLQLTE EDLETNKLDP KEVDKNLKES SDENLMEHSL KQFSGPDPLS
701 STSSSLLYPL IKLAVEATGQ QDFTQTANGQ ACLIPDVLPT QIYPLPKQQN
751 LPKRPTSLPL NTKNSTKEPR LKFGSKHKSN LKQVETGVAK MNTINAAEPH
801 VVTVTMNGVA GRNHSVNSHA ATTQYANGTV LSGQTTNIVT HRAQEMLQNQ
851 FIGEDTRLNI NSSPDEHEPL LRREQQAGHD EGVLDRLVDR RERPLEGGRT
901 NSNNNNSNPC SEQDVLAQGV PSTAADPGPS KPRRAQRPNS LDLSATNVLD
951 GSSIQIGEST QDGKSGSGEK IKKRVKTPYS LKRWRPSTWV ISTESLDCEV
1001 NNNGSNRAVH SKSSTAVYLA EGGTATTMVS KDIGMNCL

(SEQ ID NO: 148)

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

A processed extracellular BMPRII polypeptide sequence is as follows:

SQNQERLCAFKDPYQQDLGIGESRISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHI
GDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET
(SEQ ID NO: 150)

A nucleic acid sequence encoding BMPRII precursor protein is shown in SEQ ID NO: 205, as follows nucleotides 1149-4262 of Genbank Reference Sequence NM_001204.6. A nucleic acid sequence encoding an extracellular BMPRII polypeptide is shown in SEQ ID NO: 206.

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: 149)

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

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

SQNQERLCAFKDPYQQDLGIGESRISHENGTILCSKGSTCYGLWEKSKGDINLVKQGCWSHI
GDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFPPPDTTPLSPPHSFNRDET
(SEQ ID NO: 151)

A nucleic acid sequence encoding human BMPRII precursor protein (isoform 2) is shown in SEQ ID NO: 207, corresponding to nucleotides 163-1752 of Genbank Reference Sequence U25110.1. The signal sequence is underlined. A nucleic acid sequence encoding an extracellular BMPRII polypeptide (isoform 2) is shown in SEQ ID NO: 208.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one BMPRII polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, BMPRII polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising a BMPRII polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of BMPRII). In other preferred embodiments, BMPRII polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one BMPRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 148, 150, 149, 151, 152, 153, 156, or 157. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one BMPRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 148, 150, 149, 151, 152, 153, 156, or 157.

In certain aspects, the disclosure relates to a heteromultimer that comprises an BMPRII-Fc fusion protein. In some embodiments, the BMPRII-Fc fusion protein comprises an BMPRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 27-34 (e.g., amino acid residues 27, 28, 29, 30, 31, 32, 33, and 34) SEQ ID NO: 148 or 149, and ends at any one of amino acids 123-150 (e.g., amino acid residues 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150) of SEQ ID NO: 148 or 149. In some embodiments, the BMPRII-Fc fusion protein comprises an BMPRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-123 of SEQ ID NO: 148 or 149. In some embodiments, the BMPRII-Fc fusion protein comprises an BMPRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 27-150 of SEQ ID NO: 148 or 149. In some embodiments, the BMPRII-Fc fusion protein comprises an BMPRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 148, 150, 149, 151, 152, 153, 154, 155, 156, 157, 158, and 159.

The BMPRII-Fc polypeptide sequence (SEQ ID NO: 152) 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: 152)
```

The leader sequence and linker sequence are underlined. To promote formation of the BMPRII-Fc:ActRIIB-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: 152 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: 257):

```
   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: 257)
```

The mature BMPRII-Fc fusion polypeptide (SEQ ID NO: 153) 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: 153)

The BMPRII-Fc polypeptide sequence (SEQ ID NO: 156) 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: 156)

The leader sequence and linker sequence are underlined. To promote formation of the BMPRII-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 156 may optionally be provided with the lysine removed from the C-terminus.

The mature BMPRII-Fc fusion polypeptide (SEQ ID NO: 157) 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: 157)

In certain aspects, the present disclosure relates to protein complexes that comprise a TGFBRII polypeptide. As used herein, the term "TGFBRII" refers to a family of transforming growth factor-beta receptor II (TGFBRII) proteins from any species and variants derived from such proteins by mutagenesis or other modification. Reference to TGFBRII herein is understood to be a reference to any one of the currently identified forms.

Members of the TGFBRII family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

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

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

```
  1 MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL
 51 CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
101 CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS
151 EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI SVIIIFYCYR VNRQQKLSST
201 WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE LLPIELDTLV
251 GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
301 HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL
351 GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
401 SLRLDPTLSV DDLANSGQVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM
451 ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI
501 PSFWLNHQGI QMVCETLTEC WDHDPEARLT AQCVAERFSE LEHLDRLSGR
551 SCSEEKIPED GSLNTTK    (SEQ ID NO: 194)
```

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

A processed extracellular TGFBRII polypeptide sequence is as follows:

```
TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVC
VAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDN
IIFSEEYNTSNPDLLLVIFQ (SEQ ID NO: 195)
```

A nucleic acid sequence encoding TGFBRII precursor protein is shown in SEQ ID NO:196, corresponding to nucleotides 383-2083 of Genbank Reference Sequence NM_003242.5. A nucleic acid sequence encoding a processed extracellular TGFBRII polypeptide is shown in SEQ ID NO: 197.

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

1 MGRGLLRGLW PLHIVLWTRI AS**TIPPHVQK SDVEMEAQKD EIICPSCNRT**
51 **AHPLRHINND MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS**
101 **ICEKPQEVCV AVWRKNDENI TLETVCHDPK LPYHDFILED AASPKCIMKE**
151 **KKKPGETFFM CSCSSDECND NIIFSEEYNT SNPDLLLVIF Q**VTGISLLPP
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: 198)

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

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

TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFS
TCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKC
IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQ (SEQ ID NO: 199)

A nucleic acid sequence encoding the TGFBRII precursor protein (isoform A) is shown in SEQ ID NO: 202, corresponding to nucleotides 383-2158 of Genbank Reference Sequence NM_001024847.2. A nucleic acid sequence encoding the processed extracellular TGFBRII polypeptide (isoform A) is shown in SEQ ID NO: 203.

Either of the foregoing TGFβRII isoforms (SEQ ID NOs: 194, 195, 198, and 199) could incorporate an insertion of 36 amino acids (SEQ ID NO: 204) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 194; positions 129 and 130 of SEQ ID NO: 195; positions 176 and 177 of SEQ ID NO: 198; or positions 154 and 155 of SEQ ID NO: 199) 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: 204)

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one TGFBRII polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, TGFBRII polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising a TGFBRII polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of TGFBRII). In other preferred embodiments, TGFBRII polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one TGFBRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 194, 195, 198, or 199, with or without insertion of SEQ ID NO: 204 as described above. In some embodiments, heteromultimer complexes of the disclosure consist or consist essentially of at least one TGFBRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 194, 195, 198, or 199, with or without insertion of SEQ ID NO: 204.

In certain aspects, the disclosure relates to a heteromultimer that comprises an TGFBII-Fc fusion protein. In some embodiments, the TGFBII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 23-44 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44) of SEQ ID NO: 160, and ends at any one of amino acids 168-191 (e.g., 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190 or 191) of SEQ ID NO: 160. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 44-168 of SEQ ID NO: 160. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-191 of SEQ ID NO: 160. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the

amino acid sequence of any one of SEQ ID Nos: 161, 162, 160, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, and 179. In some embodiments, the TGFBII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 23-51 (e.g., 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, and 51) of SEQ ID NO: 161, and ends at any one of amino acids 143-166 (e.g., 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, and 166) of SEQ ID NO: 161. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 51-143 of SEQ ID NO: 161. In some embodiments, the TGFBRII-Fc fusion protein comprises an TGFBRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23-166 of SEQ ID NO: 161.

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

```
  1 MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL
 51 CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
101 CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS
151 EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI SVIIIFYCYR VNRQQKLSST
201 WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE LLPIELDTLV
251 GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
301 HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL
351 GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
401 SLRLDPTLSV DDLANSGQVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM
451 ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI
501 PSFWLNHQGI QMVCETLTEC WDHDPEARLT AQCVAERFSE LEHLDRLSGR
551 SCSEEKIPED GSLNTTK    (SEQ ID NO: 161)
```

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

A processed extracellular TGFBRII polypeptide sequence is as follows:

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVC
VAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDN
IIFSEEYNTSNPDLLLVIFQ (SEQ ID NO: 162)

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: 160)

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

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

TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFS
TCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKC
IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQ (SEQ ID NO: 163)

The TGFβRII$_{SHORT}$-Fc polypeptide sequence (SEQ ID NO: 164) 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: 164)

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{SHORT}$-Fc:ActRIIB-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: 164 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: 259):

```
   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: 259)
```

The mature TGFβRII$_{SHORT}$-Fc fusion polypeptide (SEQ ID NO: 165) 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: 165)
```

The TGFβRII$_{LONG}$-Fc polypeptide sequence (SEQ ID NO: 166) 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: 166)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{LONG}$-Fc:ActRIIB-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: 166 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: 260):

```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51  AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
101  ATGTGGAAAT GGAGGCCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
151  AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
```

```
 201  CAACAACGGT GCAGTCAAGT TTCCACAACT GTGTAAATTT TGTGATGTGA
 251  GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
 301  ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
 351  GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCCAAGCTCC
 401  CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
 451  AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
 501  TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
 551  ATCCTGACAC CGGTGGTGGA ACTCACACAT GCCCACCGTG CCCAGCACCT
 601  GAACTCCTGG GGGGACCGTC AGTCTTCCTC TTCCCCCCAA AACCCAAGGA
 651  CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG
 701  TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG
 751  GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC
 801  GTACCGTGTG GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG
 851  GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC
 901  GAGAAAACCA TCTCCAAAGC CAAAGGGCAG CCCCGAGAAC CACAGGTGTA
 951  CACCCTGCCC CCATCCCGGA AGGAGATGAC CAAGAACCAG GTCAGCCTGA
1001  CCTGCCTGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT GGAGTGGGAG
1051  AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGAA
1101  GTCCGACGGC TCCTTCTTCC TCTATAGCAA GCTCACCGTG GACAAGAGCA
1151  GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG
1201  CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAA
      (SEQ ID NO: 260)
```

The mature TGFβRII$_{LONG}$-Fc fusion polypeptide (SEQ ID NO: 167) 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: 167)
```

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: 172) 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: 172)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{SHORT}$-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 172 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{SHORT}$-Fc fusion polypeptide (SEQ ID NO: 173) 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: 173)
```

To guide heterodimer formation with the certain Fc fusion polypeptides disclosed herein, four amino acid substitutions can be introduced into the Fc domain of the ALK1 fusion polypeptide.

In some embodiments, the TGFβRII$_{LONG}$-Fc polypeptide sequence (SEQ ID NO: 174) is 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: 174)
```

The leader sequence and linker sequence are underlined. To promote formation of the TGFβRII$_{LONG}$-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 174 may optionally be provided with the lysine removed from the C-terminus.

The mature TGFβRII$_{LONG}$-Fc fusion polypeptide (SEQ ID NO: 175) 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: 175)
```

In certain aspects, the present disclosure relates to protein complexes that comprise an MISRII polypeptide. As used herein, the term "MISRII" refers to a family of Müllerian inhibiting substance receptor type II (MISRII) proteins from any species and variants derived from such MISRII proteins by mutagenesis or other modification. Reference to MISRII

herein is understood to be a reference to any one of the currently identified forms. Members of the MISRII family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

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

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

1 MLGSLGLWAL 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: 180)

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

A processed extracellular MISRII polypeptide sequence is as follows:

PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
RDSDEPGCESLHCDPSPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
GESIWMAL (SEQ ID NO: 183)

A nucleic acid sequence encoding the MISRII precursor protein is shown in SEQ ID NO: 209, corresponding to nucleotides 81-1799 of Genbank Reference Sequence NM_020547.2. A nucleic acid sequence encoding the extracellular human MISRII polypeptide is shown in SEQ ID NO: 210.

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: 181)

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

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

PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
RDSDEPGCESLHCDPSPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
GESIWMAL (SEQ ID NO: 184)

A nucleic acid sequence encoding the MISRII precursor protein (isoform 2) is shown in SEQ ID NO: 211, corresponding to nucleotides 81-1514 of Genbank Reference Sequence NM_001164690.1. A nucleic acid sequence encoding processed soluble (extracellular) human MISRII polypeptide (isoform 2) is shown in SEQ ID NO: 212.

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 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: 182)

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

A processed extracellular MISRII polypeptide sequence (isoform 3) is as follows:
PPNRRTCVFFEAPGVRGSTKTLGELLDTGTELPRAIRCLYSRCCFGIWNLTQDRAQVEMQGC
RDSDEPGCESLHCDPSPRAHPSPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAP
GESIWMAL (SEQ ID NO: 185)

A nucleic acid sequence encoding human MISRII precursor protein (isoform 3) is shown in SEQ ID NO: 213, corresponding to nucleotides 81-1514 of Genbank Reference Sequence NM_001164691.1. A nucleic acid sequence encoding a processed soluble (extracellular) human MISRII polypeptide (isoform 3) is shown in SEQ ID NO: 214.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one MISRII polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, MISRII polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising a MISRII polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of MISRII). In other preferred embodiments, MISRII polypeptides for use in accordance with the inventions of the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one MISRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 180, 183, 181, 184, 182, or 185. In some embodiments, heteromultimers of the disclosure consist or consist essentially of at least one MISRII polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 180, 183, 181, 184, 182, or 185.

In certain aspects, the disclosure relates to a heteromultimer that comprises an MISRII-Fc fusion protein. In some embodiments, the MISRII-Fc fusion protein comprises an MISRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence that begins at any one of amino acids 17-24 (e.g., amino acid residues 17, 18, 19, 20, 21, 22, 23, and 24) SEQ ID NO: 180, 181, or 182, and ends at any one of amino acids 116-149 (e.g., amino acid residues 116, 117, 118, 119, 120, 121, 122 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, and 149) of SEQ ID NO: 180, 181, or 182. In some

embodiments, the MISRII-Fc fusion protein comprises an MISRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 24-116 of SEQ ID NO: 180, 181, or 182. In some embodiments, the MISRII-Fc fusion protein comprises an MISRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 17-149 of SEQ ID NO: 180, 181, or 182. In some embodiments, the MISRII-Fc fusion protein comprises an MISRII domain comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID Nos: 180, 183, 181, 184, 182, 185, 186, 187, 188, 189, 190, 191, 192, and 193.

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

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

In certain embodiments, the present disclosure contemplates specific mutations of a TGF-beta superfamily type I receptor polypeptide (*e.g.*, ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) and/or a TGF-beta superfamily type II receptor polypeptide (*e.g.*, ActRIIA, ActRIIB, TGFBRII, BMPRII, and MISRII) receptor of the disclosure so as to alter the glycosylation of the polypeptide. Such mutations may be selected so as to introduce or eliminate one or more glycosylation sites, such as O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine or asparagine-X-serine (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. Removal of one or more carbohydrate moieties present on a polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of a polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* [Meth. Enzymol. (1987) 138:350]. The sequence of a polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect, and plant cells may all introduce

differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, TGF-beta superfamily type I and II receptor complexes of the present disclosure for use in humans may be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other mammalian expression cell lines are expected to be useful as well.

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

The activity of a TGF-beta superfamily heteromultimer of the disclosure also may be tested, for example in a cell-based or *in vivo* assay. For example, the effect of a heteromultimer complex on the expression of genes or the activity of proteins involved in muscle production in a muscle cell may be assessed. This may, as needed, be performed in the presence of one or more recombinant TGF-beta superfamily ligand proteins (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic

factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty), and cells may be transfected so as to produce a TGF-beta superfamily type I and II receptor complex, and optionally, a TGF-beta superfamily ligand. Likewise, a heteromultimer complex of the disclosure may be administered to a mouse or other animal, and one or more measurements, such as muscle formation and strength may be assessed using art-recognized methods. Similarly, the activity of a heteromultimer, or variants thereof, may be tested in osteoblasts, adipocytes, and/or neuronal cells for any effect on growth of these cells, for example, by the assays as described herein and those of common knowledge in the art. A SMAD-responsive reporter gene may be used in such cell lines to monitor effects on downstream signaling.

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

Many methods known in the art can be used to generate heteromultimers of the disclosure. For example, non-naturally occurring disulfide bonds may be constructed by replacing on a first polypeptide (*e.g.*, a variant ActRIIB 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.*, an unmodified ActRIIB polypeptide or a variant ActRIIB polypeptide different from that present in the first 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 variant ActRIIB polypeptide and the amino acid sequence of a first member of an interaction pair; and the second polypeptide comprises the amino acid sequence of an unmodified ActRIIB polypeptide, or a variant ActRIIB polypeptide different from that present in the first 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. 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 1B). 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 (see, e.g., Figure 1A). 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 a variant ActRIIB polypeptide or an unmodified ActRIIB polypeptide fused to a polypeptide comprising a constant domain of an immunoglobulin, such as a CH1, CH2, or CH3 domain of an immunoglobulin or an Fc domain. Fc domains derived from human IgG1, IgG2, IgG3, and IgG4 are provided herein. Other mutations are known that decrease either CDC or ADCC activity, and collectively, any of these variants are included in the disclosure and may be used as advantageous components of a heteromultimers of the disclosure. Optionally, the IgG1 Fc domain of SEQ ID NO: 13 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 variant 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 variant 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 wild-type 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: 13). 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: 13. Naturally occurring variants in G1Fc would include E134D and M136L according to the numbering system used in SEQ ID NO: 13 (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: 13)
```

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: 14). 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: 14.

```
  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: 14)
```

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: 15) contains a short hinge region consisting of a single 15-residue segment, whereas the second G3Fc sequence (SEQ ID NO: 16) 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: 15 and 16.

```
  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: 15)

  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: 16)
```

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: 15, 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: 17). 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: 17.

```
  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: 17)
```

A variety of engineered mutations in the Fc domain are presented herein with respect to the G1Fc sequence (SEQ ID NO: 13), and analogous mutations in G2Fc, G3Fc, and G4Fc can be derived from their alignment with G1Fc in Figure 4. Due to unequal hinge lengths, analogous Fc positions based on isotype alignment (Figure 4) possess different amino acid numbers in SEQ ID NOs: 13, 14, 15, and 17. 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: 13, 14, 15, 16, or 17) 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: 13), the human IgG1 heavy chain constant domain (Uniprot P01857), and the human IgG1 heavy chain is as follows.

Correspondence of C_H3 Positions in Different Numbering Systems

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

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

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

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

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 heteromultimers 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 a first variant ActRIIB polypeptide, a second variant ActRIIB polypeptide, or an unmodified ActRIIB polypeptide of the construct, with or without an optional linker, to generate a variant ActRIIB-Fc or unmodified ActRIIB-Fc fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc sequence to favor generation of the desired multichain construct (e.g., a variant ActRIIB-Fc heteromultimer). 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 a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 18 or SEQ ID NO: 19, 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 4) will generate

complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 18 and 19).

```
  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: 18)

  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: 19)
```

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 a first variant ActRIIB polypeptide, a second variant ActRIIB polypeptide, or an unmodified ActRIIB polypeptide of the construct, with or without an optional linker, to generate a variant ActRIIB-Fc or unmodified ActRIIB-Fc fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc sequence to favor generation of the desired multichain construct. In this example based on knobs-into-holes pairing, SEQ ID NO: 20 [human G1Fc(T144Y)] and SEQ ID NO: 21 [human G1Fc(Y185T)] are examples of complementary Fc sequences in which the engineered amino acid substitutions are double underlined, and a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 20 or SEQ ID NO: 21, 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 4) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 20 and 21).

```
  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: 20)

  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: 21)
```

An example of Fc complementarity based on knobs-into-holes pairing combined with an engineered disulfide bond is disclosed in SEQ ID NO: 22 [hG1Fc(S132C/T144W)] and SEQ ID NO: 23 [hG1Fc(Y127C/T144S/L146A/Y185V)]. The engineered amino acid substitutions in these sequences are double underlined, and a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 22 or SEQ ID NO: 23, 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 4) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 22 and 23).

```
  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: 22)

  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: 23)
```

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 a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB polypeptide of the construct, with

or without an optional linker, to generate a variant ActRIIB-Fc or unmodified ActRIIB-Fc fusion polypeptide. This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc sequence to favor generation of the desired multichain construct. In this example based on SEEDbody (Sb) pairing, SEQ ID NO: 24 [hG1Fc(Sb_{AG})] and SEQ ID NO: 25 [hG1Fc(Sb_{GA})] are examples of complementary IgG Fc sequences in which the engineered amino acid substitutions from IgA Fc are double underlined, and a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 24 or SEQ ID NO: 25, 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 4) will generate an Fc monomer which may be used in the complementary IgG-IgA pair below (SEQ ID NOs: 24 and 25).

```
  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: 24)

  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: 25)
```

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 a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB polypeptide of the construct, with or without an optional linker, to generate a variant ActRIIB-Fc or unmodified ActRIIB-Fc 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: 26 [hG1Fc-Ap1 (acidic)] and SEQ ID NO: 27 [hG1Fc-Bp1 (basic)] are examples of complementary IgG Fc sequences in which the engineered complimentary leucine zipper sequences are underlined, and a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or wild-type ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 26 or SEQ ID NO: 27, 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 4) will generate an Fc monomer which may be used in the complementary leucine zipper-forming pair below (SEQ ID NOs: 26 and 27).

```
  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: 26)

  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: 27)
```

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains by methods described above in combination with additional mutations in the Fc domain which facilitate purification of the desired heteromeric species. An example uses complementarity of Fc domains based on knobs-into-holes pairing combined with an engineered disulfide bond, as disclosed in SEQ ID NOs: 22 and 23, plus additional substitution of two negatively charged amino acids (aspartic acid or glutamic acid) in one Fc-containing polypeptide chain and two positively charged amino acids (e.g., arginine) in the complementary Fc-containing polypeptide chain (SEQ ID NOs: 28-29). These four amino acid substitutions facilitate selective purification of the desired heteromeric fusion protein from a heterogeneous polypeptide mixture based on differences in isoelectric point or net molecular charge. The engineered amino acid substitutions in these sequences are double

underlined below, and a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 28 or SEQ ID NO: 29, 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 4) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 28-29).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTENQ VSLWCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQDSLS LSPGK      (SEQ ID NO: 28)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSREEMTKNQ VSLSCAVKGF
151  YPSDIAVEWE SRGQPENNYK TTPPVLDSRG SFFLVSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK      (SEQ ID NO: 29)
```

Another example involves complementarity of Fc domains based on knobs-into-holes pairing combined with an engineered disulfide bond, as disclosed in SEQ ID NOs: 22-23, plus a histidine-to-arginine substitution at position 213 in one Fc-containing polypeptide chain (SEQ ID NO: 30). This substitution (denoted H435R in the numbering system of Kabat et al.) facilitates separation of desired heteromer from undesirable homodimer based on differences in affinity for protein A. The engineered amino acid substitution is indicated by double underline, and a first variant ActRIIB polypeptide, second variant ActRIIB polypeptide, or unmodified ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 30 or SEQ ID NO: 23, 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 4) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair of SEQ ID NO: 30 (below) and SEQ ID NO: 23.

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNRYTQKSLS LSPGK     (SEQ ID NO: 30)
```

A variety of engineered mutations in the Fc domain are presented above with respect to the G1Fc sequence (SEQ ID NO: 13). Analogous mutations in G2Fc, G3Fc, and G4Fc can be derived from their alignment with G1Fc in Figure 4. Due to unequal hinge lengths, analogous Fc positions based on isotype alignment (Figure 4) possess different amino acid numbers in SEQ ID NOs: 13, 14, 15, 16, and 17 as summarized in the following table.

Correspondence between C_H3 Positions for Human Fc Isotypes*			
IgG1	IgG4	IgG2	IgG3
SEQ ID NO: 13	SEQ ID NO: 17	SEQ ID NO: 14	SEQ ID NO: 15
Numbering begins at THT…	Numbering begins at ESK…	Numbering begins at VEC…	Numbering begins at EPK…
Y127	Y131	Y125	Y134
S132	S136	S130	S139
E134	E138	E132	E141
K138	K142	K136	K145
T144	T148	T142	T151
L146	L150	L144	L153
N162	N166	N160	S169
K170	K174	K168	N177
D177	D181	D175	D184
D179	D183	D177	D186
Y185	Y189	Y183	Y192
K187	R191	K185	K194
H213	H217	H211	R220
K217	K221	K215	K224
* Numbering based on multiple sequence alignment shown in Figure 4			

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, an ActRIIB polypeptide domain may be placed C-terminal to a heterologous domain, or alternatively, a heterologous domain may be placed C-terminal to an ActRIIB polypeptide domain. The ActRIIB 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 ActRIIB polypeptide may comprise an amino acid sequence as set forth in the formula A-B-C. The B portion corresponds to an ActRIIB 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: 261), GGGG (SEQ ID NO: 262), TGGGG (SEQ ID NO: 263), SGGGG (SEQ ID NO: 264), TGGG (SEQ ID NO: 265), or SGGG (SEQ ID NO: 266) singlets, or repeats. In certain embodiments, an ActRIIB 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 an ActRIIB 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, an ActRIIB 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 an ActRIIB polypeptide domain, and C is an immunoglobulin Fc domain.

In certain embodiments, the variant ActRIIB polypeptides of the present invention contain one or more modifications that are capable of stabilizing the variant ActRIIB polypeptides. For example, such modifications enhance the in vitro half life of the variant ActRIIB polypeptides, enhance circulatory half life of the variant ActRIIB polypeptides or reducing proteolytic degradation of the variant ActRIIB polypeptides. Such stabilizing modifications include, but are not limited to, fusion proteins (including, for example, fusion proteins comprising a variant ActRIIB polypeptide and a stabilizer domain), modifications of a glycosylation site (including, for example, addition of a glycosylation site to a variant ActRIIB polypeptide), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from a variant ActRIIB polypeptide). In the case of fusion proteins, a variant ActRIIB polypeptide is fused to a stabilizer domain such as an IgG molecule (e.g., an Fc domain). As used herein, the term "stabilizer domain" not only refers to a fusion domain (e.g., Fc) as in the case of fusion proteins, but also includes

nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous polymer, such as polyethylene glycol.

In certain embodiments, the present invention makes available isolated and/or purified forms of the variant ActRIIB polypeptides, which are isolated from, or otherwise substantially free of, other proteins.

In certain embodiments, ActRIIB polypeptides (unmodified or modified) of the invention can be produced by a variety of art-known techniques. For example, such ActRIIB polypeptides 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 (e.g., Advanced ChemTech Model 396; Milligen/Biosearch 9600). Alternatively, the ActRIIB polypeptides, fragments or variants thereof may be recombinantly produced using various expression systems (e.g., E. coli, Chinese Hamster Ovary cells, COS cells, baculovirus) as is well known in the art (also see below). In a further embodiment, the modified or unmodified ActRIIB polypeptides may be produced by digestion of naturally occurring or recombinantly produced full-length ActRIIB 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. Alternatively, such ActRIIB polypeptides may be produced from naturally occurring or recombinantly produced full-length ActRIIB polypeptides such as standard techniques known in the art, such as by chemical cleavage (e.g., cyanogen bromide, hydroxylamine).

3. Nucleic Acids Encoding ActRIIB Polypeptides

In certain aspects, the invention provides isolated and/or recombinant nucleic acids encoding any of the ActRIIB polypeptides (e.g., soluble ActRIIB polypeptides), including any of the variants disclosed herein. For example, SEQ ID NO: 4 encodes a naturally occurring ActRIIB precursor polypeptide, while SEQ ID NO: 3 encodes a soluble ActRIIB polypeptide. The subject nucleic acids may be single-stranded or double stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids are may be used, for

example, in methods for making ActRIIB polypeptides or as direct therapeutic agents (e.g., in a gene therapy approach).

In certain aspects, the subject nucleic acids encoding ActRIIB polypeptides are further understood to include nucleic acids that are variants of SEQ ID NO: 3. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants; and will, therefore, include coding sequences that differ from the nucleotide sequence of the coding sequence designated in SEQ ID NO: 4.

In certain embodiments, the invention provides isolated or recombinant nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 3. One of ordinary skill in the art will appreciate that nucleic acid sequences complementary to SEQ ID NO: 3, and variants of SEQ ID NO: 3 are also within the scope of this invention. In further embodiments, the nucleic acid sequences of the invention can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

In other embodiments, nucleic acids of the invention also include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequence designated in SEQ ID NO: 3, complement sequence of SEQ ID NO: 3, or fragments thereof. As discussed above, one of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. 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 invention 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 NO: 3 due to degeneracy in the genetic code are also within the scope of the invention. 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 invention.

In certain embodiments, the recombinant nucleic acids of the invention 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 invention. 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 a preferred embodiment, 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 invention, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding an ActRIIB polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the variant ActRIIB 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 variant ActRIIB polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda , the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

A recombinant nucleic acid of the invention 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 variant ActRIIB polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the 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 *Molecular Cloning A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17. 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 variant ActRIIB 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 variant ActRIIB polypeptides in cells propagated in culture, e.g., to produce proteins, including fusion proteins or variant proteins, for purification.

This invention also pertains to a host cell transfected with a recombinant gene including a coding sequence (e.g., SEQ ID NO: 4) for one or more of the subject variant ActRIIB polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a variant ActRIIB polypeptide of the invention may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art.

Accordingly, the present invention further pertains to methods of producing the subject variant ActRIIB polypeptides. For example, a host cell transfected with an expression vector encoding an ActRIIB polypeptide can be cultured under appropriate conditions to allow expression of the ActRIIB polypeptide to occur. The ActRIIB polypeptide may be secreted and isolated from a mixture of cells and medium containing the ActRIIB polypeptide. Alternatively, the ActRIIB polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art. The subject ActRIIB 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, and immunoaffinity purification with antibodies specific for

particular epitopes of the ActRIIB polypeptides. In a preferred embodiment, the variant ActRIIB polypeptide is a fusion protein containing a domain which facilitates its purification.

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 variant ActRIIB 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 variant ActRIIB polypeptide (e.g., see Hochuli et al., (1987) *J. Chromatography* 411:177; and Janknecht et al., *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, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al., John Wiley & Sons: 1992).

4. Screening Assays

In certain aspects, the present invention relates to the use of the subject variant ActRIIB polypeptides (e.g., soluble ActRIIB polypeptides) to identify compounds (agents) which are agonist or antagonists of the variant ActRIIB polypeptides. Compounds identified through this screening can be tested in tissues such as bone, cartilage, muscle, fat, and/or neurons, to assess their ability to modulate tissue growth in vitro. Optionally, these compounds can further be tested in animal models to assess their ability to modulate tissue growth in vivo.

There are numerous approaches to screening for therapeutic agents for modulating tissue growth by targeting the variant ActRIIB polypeptides. In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb

ActRIIB-mediated effects on growth of bone, cartilage, muscle, fat, and/or neurons. In certain embodiments, the assay is carried out to screen and identify compounds that specifically inhibit or reduce binding of an ActRIIB polypeptide to its binding partner, such as an ActRIIB ligand (e.g., activin, Nodal, GDF8, GDF11 or BMP7). Alternatively, the assay can be used to identify compounds that enhance binding of an ActRIIB polypeptide to its binding protein such as an ActRIIB ligand. In a further embodiment, the compounds can be identified by their ability to interact with an ActRIIB polypeptide.

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 a specific embodiment, the test agent is a small organic molecule having a molecular weight of less than about 2,000 daltons.

The test compounds of the invention 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 an ActRIIB polypeptide and its binding protein (e.g., an ActRIIB ligand).

Merely to illustrate, in an exemplary screening assay of the present invention, the compound of interest is contacted with an isolated and purified ActRIIB polypeptide which is ordinarily capable of binding to an ActRIIB ligand, as appropriate for the intention of the assay. To the mixture of the compound and ActRIIB polypeptide is then added a composition containing an ActRIIB ligand. Detection and quantification of ActRIIB/ActRIIB ligand complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the ActRIIB polypeptide 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 ActRIIB ligand is added to a composition containing the ActRIIB polypeptide, and the formation of ActRIIB/ActRIIB 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.

Complex formation between the ActRIIB polypeptide and its binding 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 ActRIIB polypeptide or its binding protein, by immunoassay, or by chromatographic detection.

In certain embodiments, the present invention 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 an ActRIIB polypeptide and its binding protein. Further, other modes of detection, such as those based on optical waveguides (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 invention.

Moreover, the present invention contemplates the use of an interaction trap assay, also known as the "two hybrid assay," for identifying agents that disrupt or potentiate interaction between an ActRIIB polypeptide and its binding protein. See for example, 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 invention contemplates the use of reverse two hybrid systems to identify compounds (e.g., small molecules or peptides) that dissociate interactions between an ActRIIB polypeptide and its binding protein. See for example, 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 variant ActRIIB polypeptide of the invention. The interaction between the compound and the variant ActRIIB polypeptide 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 (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 variant ActRIIB polypeptide. This may include a solid phase or fluid phase binding event. Alternatively, the gene encoding a variant ActRIIB polypeptide 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 a 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 or fluorescence or surface plasmon resonance.

In certain aspects, the present invention provides methods and agents for stimulating muscle growth and increasing muscle mass, for example, by antagonizing functions of an ActRIIB polypeptide and/or an ActRIIB ligand. Therefore, any compound identified can be tested in whole cells or tissues, in vitro or in vivo, to confirm their ability to modulate muscle

growth. Various methods known in the art can be utilized for this purpose. For example, methods of the invention are performed such that the signal transduction through an ActRIIB protein activated by binding to an ActRIIB ligand (e.g., GDF8) has been reduced or inhibited. It will be recognized that the growth of muscle tissue in the organism would result in an increased muscle mass in the organism as compared to the muscle mass of a corresponding organism (or population of organisms) in which the signal transduction through an ActRIIB protein had not been so effected.

For example, the effect of the variant ActRIIB polypeptides or test compounds on muscle cell growth/proliferation can be determined by measuring gene expression of Pax-3 and Myf-5 which are associated with proliferation of myogenic cells, and gene expression of MyoD which is associated with muscle differentiation (e.g., Amthor et al., Dev Biol. 2002, 251:241-57). It is known that GDF8 down-regulates gene expression of Pax-3 and Myf-5, and prevents gene expression of MyoD. The variant ActRIIB polypeptides or test compounds are expected to antagonize this activity of GDF8. Another example of cell-based assays includes measuring the proliferation of myoblasts such as C(2)C(12) myoblasts in the presence of the ActRIIB polypeptides or test compounds (e.g., Thomas et al., J Biol Chem. 2000, 275:40235-43).

The present invention also contemplates in vivo assays to measure muscle mass and strength. For example, Whittemore et al. (Biochem Biophys Res Commun. 2003, 300:965-71) discloses a method of measuring increased skeletal muscle mass and increased grip strength in mice. Optionally, this method can be used to determine therapeutic effects of test compounds (e.g., variant ActRIIB polypeptides) on muscle diseases or conditions, for example those diseases for which muscle mass is limiting.

In certain aspects, the present invention provides methods and agents for modulating (stimulating or inhibiting) bone formation and increasing bone mass. Therefore, any compound identified can be tested in whole cells or tissues, in vitro or in vivo, to confirm their ability to modulate bone or cartilage growth. Various methods known in the art can be utilized for this purpose.

For example, the effect of the variant ActRIIB polypeptides or test compounds on bone or cartilage growth can be determined by measuring induction of Msx2 or differentiation of osteoprogenitor cells into osteoblasts in cell based assays (see, e.g., Daluiski et al., Nat Genet. 2001, 27(1):84-8; Hino et al., Front Biosci. 2004, 9:1520-9). Another

example of cell-based assays includes analyzing the osteogenic activity of the subject ActRIIB polypeptides and test compounds in mesenchymal progenitor and osteoblastic cells. To illustrate, recombinant adenoviruses expressing an ActRIIB polypeptide were constructed to infect pluripotent mesenchymal progenitor C3H10T1/2 cells, preosteoblastic C2C12 cells, and osteoblastic TE-85 cells. Osteogenic activity is then determined by measuring the induction of alkaline phosphatase, osteocalcin, and matrix mineralization (see, e.g., Cheng et al., J bone Joint Surg Am. 2003, 85-A(8):1544-52).

The present invention also contemplates in vivo assays to measure bone or cartilage growth. For example, Namkung-Matthai et al., Bone, 28:80-86 (2001) discloses a rat osteoporotic model in which bone repair during the early period after fracture is studied. Kubo et al., Steroid Biochemistry & Molecular Biology, 68:197-202 (1999) also discloses a rat osteoporotic model in which bone repair during the late period after fracture is studied. These references are incorporated by reference herein in their entirety for their disclosure of rat model for study on osteoporotic bone fracture. In certain aspects, the present invention makes use of fracture healing assays that are known in the art. These assays include fracture technique, histological analysis, and biomechanical analysis, which are described in, for example, U.S. Pat. No. 6,521,750, which is incorporated by reference in its entirety for its disclosure of experimental protocols for causing as well as measuring the extent of fractures, and the repair process.

In certain aspects, the present invention provides methods and agents for controlling weight gain and obesity. At the cellular level, adipocyte proliferation and differentiation is critical in the development of obesity, which leads to the generation of additional fat cells (adipocytes). Therefore, any compound identified can be tested in whole cells or tissues, in vitro or in vivo, to confirm their ability to modulate adipogenesis by measuring adipocyte proliferation or differentiation. Various methods known in the art can be utilized for this purpose. For example, the effect of a variant ActRIIB polypeptide (e.g., a soluble ActRIIB polypeptide) or test compounds on adipogenesis can be determined by measuring differentiation of 3T3-L1 preadipocytes to mature adipocytes in cell based assays, such as, by observing the accumulation of triacylglycerol in Oil Red O staining vesicles and by the appearance of certain adipocyte markers such as FABP (aP2/422) and PPARγ2. See, for example, Reusch et al., 2000, Mol Cell Biol. 20:1008-20; Deng et al., 2000, Endocrinology. 141:2370-6; Bell et al., 2000, Obes Res. 8:249-54. Another example of cell-based assays includes analyzing the role of variant ActRIIB polypeptides and test compounds in

proliferation of adipocytes or adipocyte precursor cells (e.g., 3T3-L1 cells), such as, by monitoring bromodeoxyuridine (BrdU)-positive cells. See, for example, Pico et al., 1998, Mol Cell Biochem. 189:1-7; Masuno et al., 2003, Toxicol Sci. 75:314-20.

It is understood that the screening assays of the present invention apply to not only the subject ActRIIB polypeptides and variants of the ActRIIB polypeptides, but also any test compounds including agonists and antagonist of the ActRIIB polypeptides. Further, these screening assays are useful for drug target verification and quality control purposes.

6. Exemplary Therapeutic Uses

In certain embodiments, compositions of the present invention (e.g., variant ActRIIB proteins in either homomeric or heteromeric forms) can be used for treating or preventing a disease or condition that is associated with abnormal activity of ActRIIB and/or an ActRIIB ligand (e.g., GDF8 or GDF11). These diseases, disorders or conditions are generally referred to herein as "ActRIIB-associated conditions." In certain embodiments, the present invention provides methods of treating or preventing an individual in need thereof through administering to the individual a therapeutically effective amount of a variant ActRIIB protein as described above. These methods are particularly aimed at therapeutic and prophylactic treatments of animals, and more particularly, humans. The terms "subject," an "individual," or a "patient" are interchangeable throughout the specification and generally refer to mammals. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats).

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 prophylaxis of the named condition or amelioration or elimination of the condition once it has been established.

Endogenous complexes between ActRIIB and ActRIIB ligands 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,

ActRIIB-associated conditions include abnormal tissue growth and developmental defects. In addition, ActRIIB-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 ActRIIB-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, and bone degenerative disease (e.g., osteoporosis). Other exemplary ActRIIB-associated conditions include anemia, musculodegenerative and neuromuscular disorders, tissue repair (e.g., wound healing), neurodegenerative diseases (e.g., amyotrophic lateral sclerosis), immunologic disorders (e.g., disorders related to abnormal proliferation or function of lymphocytes), and obesity or disorders related to abnormal proliferation of adipocytes.

In certain embodiments, compositions of the invention (e.g., variant ActRIIB proteins) 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 variant ActRIIB proteins 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 broken. 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 insufficient in quantity or poor in quality. Having some dystrophin protects the muscles of those with BMD from degenerating as badly or as quickly as those of people with DMD.

For example, studies demonstrate that blocking or eliminating function of GDF8 in vivo can effectively treat at least certain symptoms in DMD and BMD patients. Thus, the subject variant ActRIIB proteins may act as GDF8 inhibitors (antagonists), and constitute an alternative means of blocking the functions of GDF8 and/or ActRIIB in vivo in DMD and BMD patients.

Similarly, the subject variant ActRIIB proteins provide an effective means to increase muscle mass in other disease conditions that are in need of muscle growth. For example, ALS, also called Lou Gehrig's disease (motor neuron disease) is a chronic, incurable, and unstoppable CNS disorder that attacks the motor neurons, components of the CNS that connect the brain to the skeletal muscles. In ALS, the motor neurons deteriorate and eventually die, and though a person's brain normally remains fully functioning and alert, the command to move never reaches the muscles. Most people who get ALS are between 40 and 70 years old. The first motor neurons that weaken are those leading to the arms or legs. Those with ALS may have trouble walking, they may drop things, fall, slur their speech, and laugh or cry uncontrollably. Eventually the muscles in the limbs begin to atrophy from disuse. This muscle weakness will become debilitating and a person will need a wheel chair or become unable to function out of bed. Most ALS patients die from respiratory failure or from complications of ventilator assistance like pneumonia, 3-5 years from disease onset.

Variant ActRIIB protein-induced increased muscle mass might also benefit those suffering from muscle wasting diseases. Gonzalez-Cadavid et al. (supra) reported that 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 variant ActRIIB proteins 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. Progressive weight loss in cancer anorexia-cachexia syndrome is a common feature of many types of cancer 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 without weight loss. Associated with anorexia, fat and muscle tissue wasting, psychological distress, and a lower quality of life, cachexia arises from a complex interaction between the cancer and the host. It is one of the most common causes of death among cancer patients and is present in 80% at death. It is a complex example of metabolic chaos effecting protein, carbohydrate, and fat metabolism. Tumors produce both direct and indirect abnormalities, resulting in anorexia and weight loss. Currently, there is no treatment to control or reverse the process. Cancer anorexia-cachexia syndrome 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. Cachexia should be suspected in patients with cancer if an involuntary weight loss of greater than five percent of premorbid weight occurs within a six-month period.

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 variant ActRIIB proteins as pharmaceutical compositions can be beneficially used to prevent, treat, or alleviate the symptoms of the cachexia syndrome, where muscle growth is desired.

In other embodiments, the present invention provides methods of inducing bone and/or cartilage formation, preventing bone loss, increasing bone mineralization or preventing the demineralization of bone. For example, the subject variant ActRIIB proteins have application in treating osteoporosis and the healing of bone fractures and cartilage

defects in humans and other animals. Variant ActRIIB proteins may be useful in patients that are diagnosed with subclinical low bone density, as a protective measure against the development of osteoporosis.

In one specific embodiment, methods and compositions of the present invention 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 contributes to the repair of congenital, trauma-induced, or oncologic resection induced craniofacial defects, and also is 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, the subject variant ActRIIB proteins may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. Variant ActRIIB proteins of the invention may also be useful in the treatment of osteoporosis. Further, variant ActRIIB proteins may be used in cartilage defect repair and prevention/reversal of osteoarthritis.

In another specific embodiment, the invention 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. WO84/01106. Such compositions comprise a therapeutically effective amount of at least one of the variant ActRIIB proteins of the invention in admixture with a pharmaceutically acceptable vehicle, carrier or matrix.

In another specific embodiment, methods and variant ActRIIB proteins of the invention 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. Many people know 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 a condition known as Cushing's disease, the excess amount of cortisol produced by the body results in

osteoporosis and fractures. The most common medications associated with secondary osteoporosis are the corticosteroids, a class of drugs that act like cortisol, a hormone produced naturally by the adrenal glands. Although adequate levels of thyroid hormones (which are produced by the thyroid gland) 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. Gum disease causes bone loss because these harmful bacteria in our mouths force our bodies to defend against them. The bacteria produce toxins and enzymes under the gum-line, causing a chronic infection.

In a further embodiment, the variant ActRIIB proteins of the present invention provide methods and therapeutic agents for treating diseases or disorders associated with abnormal or unwanted bone growth. For example, patients having the disease known as fibrodysplasia ossificans progressiva (FOP) grow an abnormal "second skeleton" that prevents any movement. 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 other embodiments, variant ActRIIB proteins of the present invention provide compositions and methods 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 invention relates to regulating body weight by administering to an animal (e.g., a human) in need thereof a variant ActRIIB protein.

In one specific embodiment, the present invention relates to methods and compounds 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 certain aspects, variant ActRIIB proteins 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 variant ActRIIB protein 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 variant ActRIIB protein of the present disclosure can be used to treat or prevent an anemia in a subject in need thereof. In certain embodiments, a variant ActRIIB protein 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 variant ActRIIB protein 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.

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.

In general, treatment or prevention of a disease or condition as described in the present disclosure is achieved by administering one or more variant ActRIIB proteins 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, one or more variant ActRIIB proteins of the disclosure, 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 one or more variant ActRIIB proteins to increase red blood cell levels, and then blood is drawn and stored for later use in transfusions.

One or more variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins, 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 variant ActRIIB proteins, 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins of the disclosure, optionally in combination with an EPO receptor activator. In other embodiments, a patient suffering from MDS may be treated using a combination of one or more variant ActRIIB proteins 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, a variant ActRIIB protein 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins of the disclosure and a EPO receptor activator. In certain embodiments, one or more variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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, an antagonist 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins of the disclosure, then onset of administration of the one or more variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins of the disclosure. Alternatively, a therapeutic regimen may be developed for the patient that combines one or more variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 antagonist-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 antagonist of the disclosure. A patient's baseline values for one or more hematologic parameters prior to treatment with one or more variant ActRIIB proteins 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 antagonists of the disclosure.

In certain embodiments, one or more hematologic parameters are measured in patients who are being treated with a one or more variant ActRIIB proteins 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 antagonists of the disclosure or additional dosing with another therapeutic agent. For example, if administration of one or more variant ActRIIB proteins 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 variant ActRIIB proteins of the disclosure may be reduced in amount or frequency in order to decrease the effects of the one or more variant ActRIIB proteins of the disclosure on the one or more hematologic parameters. If administration of one or more variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins of the disclosure has elevated blood pressure, then dosing with the one or more variant ActRIIB proteins 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 variant ActRIIB proteins 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 variant ActRIIB proteins of the disclosure may be terminated and the patient may be treated with a blood pressure-lowering agent.

7. Pharmaceutical Compositions

In certain embodiments, compounds of the present invention (e.g., variant ActRIIB proteins in either homomeric or heteromeric forms) are formulated with a pharmaceutically acceptable carrier. For example, a variant ActRIIB protein can be administered alone or as a component of a pharmaceutical formulation (therapeutic composition). The subject compounds may be formulated for administration in any convenient way for use in human or veterinary medicine.

In certain embodiments, the therapeutic method of the invention includes administering the composition topically, systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to a target tissue site (e.g., bone, cartilage, muscle, fat or neurons), for example, a site having a tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than the variant ActRIIB proteins which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the subject compounds (e.g., variant ActRIIB proteins) in the methods of the invention.

In certain embodiments, compositions of the present invention may include a matrix capable of delivering one or more therapeutic compounds (e.g., variant ActRIIB proteins) to a target tissue site, 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 the variant ActRIIB proteins. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on 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, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are non-biodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

In certain embodiments, variant ActRIIB proteins of the invention can be administered orally, e.g., in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of an agent as an active ingredient. An agent may also be administered as a bolus, electuary or paste.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), one or more variant ActRIIB proteins of the present invention may be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid

compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol, and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Certain compositions disclosed herein may be administered topically, either to skin or to mucosal membranes. The topical formulations may further include one or more of the wide variety of agents known to be effective as skin or stratum corneum penetration enhancers. Examples of these are 2-pyrrolidone, N-methyl-2-pyrrolidone, dimethylacetamide, dimethylformamide, propylene glycol, methyl or isopropyl alcohol, dimethyl sulfoxide, and azone. Additional agents may further be included to make the formulation cosmetically acceptable. Examples of these are fats, waxes, oils, dyes, fragrances, preservatives, stabilizers, and surface active agents. Keratolytic agents such as those known in the art may also be included. Examples are salicylic acid and sulfur.

Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required. The ointments, pastes, creams and gels may contain, in addition to a subject compound of the invention (e.g., a variant ActRIIB protein), excipients, such as animal and vegetable fats, oils, waxes,

paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a subject compound, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates, and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

In certain embodiments, pharmaceutical compositions suitable for parenteral administration may comprise one or more variant ActRIIB proteins 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, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The compositions of the invention may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as 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 subject compounds of the invention (e.g., variant ActRIIB proteins). The various factors will depend upon the disease to be treated. In the case of muscle disorders, factors may include, but are not limited to,

amount of muscle mass desired to be formed, the muscles most affected by disease, the condition of the deteriorated muscle, the patient's age, sex, and diet, time of administration, and other clinical factors. The addition of other known growth factors to the final composition, may also affect the dosage. Progress can be monitored by periodic assessment of muscle growth and/or repair, for example, by strength testing, MRI assessment of muscle size and analysis of muscle biopsies.

In certain embodiments of the invention, one or more variant ActRIIB proteins can be administered, together (simultaneously) or at different times (sequentially or overlapping). In addition, variant ActRIIB proteins can be administered with another type of therapeutic agents, for example, a cartilage-inducing agent, a bone-inducing agent, a muscle-inducing agent, a fat-reducing, or a neuron-inducing agent. The two types of compounds may be administered simultaneously or at different times. It is expected that the variant ActRIIB proteins of the invention may act in concert with or perhaps synergistically with another therapeutic agent.

In a specific example, a variety of osteogenic, cartilage-inducing and bone-inducing factors have been described, particularly bisphosphonates. See e.g., European Patent Application Nos. 148,155 and 169,016. For example, other factors that can be combined with the subject variant ActRIIB proteins include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

In certain embodiments, the present invention also provides gene therapy for the in vivo production of variant ActRIIB proteins. Such therapy would achieve its therapeutic effect by introduction of the variant ActRIIB polynucleotide sequences into cells or tissues having the disorders as listed above. Delivery of variant ActRIIB polynucleotide sequences can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Preferred for therapeutic delivery of variant ActRIIB polynucleotide sequences 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, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is 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 the variant ActRIIB polynucleotide. In one preferred embodiment, the vector is targeted to bone, cartilage, muscle or neuron cells/tissues.

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 variant ActRIIB polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention 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., Trends Biochem. Sci., 6:77, 1981). Methods for efficient gene transfer using a liposome vehicle, are known in the art, see e.g., Mannino, et al., Biotechniques, 6:682, 1988. The composition of the liposome is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Illustrative phospholipids include 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 fusion protein

Applicants constructed a soluble ActRIIB fusion protein that has the extracellular domain of human ActRIIB fused to a human G1Fc domain with a minimal linker (three glycine amino acids) in between. The construct is referred to as ActRIIB-G1Fc.

ActRIIB-G1Fc is shown below in SEQ ID NO: 5 (with the linker underlined) as purified from CHO cell lines:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDD
FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 5)

The ActRIIB-G1Fc protein was expressed in CHO cell lines. Three different leader sequences were considered:

(i) Honey bee mellitin (HBML): MKFLVNVALVFMVVYISYIYA (SEQ ID NO: 7)

(ii) Tissue plasminogen activator (TPA): MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 8)

(iii) Native: MTAPWVALALLWGSLCAG (SEQ ID NO: 9).

The selected form employs the TPA leader and has the following unprocessed amino acid sequence:

MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKR
LHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPE
AGGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA

LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 6)

This polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 10):

A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT GTGTGGAGCA GTCTTCGTTT
CGCCCGGCGC CTCTGGGCGT GGGGAGGCTG AGACACGGGA GTGCATCTAC TACAACGCCA
ACTGGGAGCT GGAGCGCACC AACCAGAGCG GCCTGGAGCG CTGCGAAGGC GAGCAGGACA
AGCGGCTGCA CTGCTACGCC TCCTGGCGCA ACAGCTCTGG CACCATCGAG CTCGTGAAGA
AGGGCTGCTG GCTAGATGAC TTCAACTGCT ACGATAGGCA GGAGTGTGTG GCCACTGAGG
AGAACCCCCA GGTGTACTTC TGCTGCTGTG AAGGCAACTT CTGCAACGAG CGCTTCACTC
ATTTGCCAGA GGCTGGGGGC CCGGAAGTCA CGTACGAGCC ACCCCCGACA GCCCCCACCG
GTGGTGGAAC TCACACATGC CCACCGTGCC CAGCACCTGA ACTCCTGGGG GGACCGTCAG
TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA
CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT
ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA
AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA
AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT
CCGACGGCTC CTTCTTCCTC TATAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG
GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA
GCCTCTCCCT GTCTCCGGGT AAATGA (SEQ ID NO: 10)

N-terminal sequencing of the CHO-cell produced material revealed a major sequence of –GRGEAE (SEQ ID NO: 11). Notably, other constructs reported in the literature begin with an –SGR… sequence.

Purification 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.

The ActRIIB-Fc fusion protein was also expressed in HEK293 cells and COS cells. Although material from all cell lines and reasonable culture conditions provided protein with

muscle-building activity in vivo, variability in potency was observed perhaps relating to cell line selection and/or culture conditions.

Example 2: Generation of Variant ActRIIB-Fc Proteins

Applicants generated a series of mutations (sequence variations) in the extracellular domain of ActRIIB and produced these variant polypeptides as soluble homodimeric fusion proteins comprising a variant ActRIIB extracellular domain and an Fc domain joined by an optional linker. The background ActRIIB-Fc fusion was ActRIIB-G1Fc as shown in SEQ ID NO: 5

Various substitution mutations were introduced into the background ActRIIB-Fc protein. Based on the data presented in Example 1, it is expected that these constructs, if expressed with a TPA leader, will lack the N-terminal serine. Mutations were generated in the ActRIIB extracellular domain by PCR mutagenesis. After PCR, fragments were purified through a Qiagen column, digested with SfoI and AgeI and gel purified. These fragments were ligated into expression vector pAID4 (see WO2006/012627) such that upon ligation it created fusion chimera with human IgG1. Upon transformation into E. coli DH5 alpha, colonies were picked and DNAs were isolated. For murine constructs (mFc), a murine IgG2a was substituted for the human IgG1. All mutants were sequence verified.

The amino acid sequence of unprocessed ActRIIB(K55A)-G1Fc is shown below (SEQ ID NO: 31). The signal sequence and linker sequence are indicated by solid underline, and the K55A substitution is indicated by double underline. The amino acid sequence of SEQ ID NO:31 may optionally be provided with the lysine removed from the C-terminus.

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD ARLHCYASWR 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 NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK   (SEQ ID NO: 31)
```

This ActRIIB(K55A)-G1Fc fusion polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 32):

```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
```

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  51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
 101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
 151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC GCCCGGCTGC 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 CATCCCGGGA GGAGATGACC AAGAACCAGG
 851  TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
 951  CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCCCCGGG
1101  TAAA    (SEQ ID NO: 32)
```

The mature ActRIIB(K55A)-G1Fc fusion polypeptide (SEQ ID NO: 33) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
   1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDARLHC 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 GQPENNYKTT PPVLDSDGSF
 301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
      (SEQ ID NO: 33)
```

The amino acid sequence of unprocessed ActRIIB(K55E)-G1Fc is shown below (SEQ ID NO: 34). The signal sequence and linker sequence are indicated by solid underline, and the K55E substitution is indicated by double underline. The amino acid sequence of SEQ ID NO:34 may optionally be provided with the lysine removed from the C-terminus.

```
   1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
  51  GLERCEGEQD ERLHCYASWR 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 NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
 351  EALHNHYTQK SLSLSPGK   (SEQ ID NO: 34)
```

This ActRIIB(K55E)-G1Fc fusion polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 35):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
 101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
 151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC GAGCGGCTGC 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 CATCCCGGGA GGAGATGACC AAGAACCAGG
 851  TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
 951  CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCCCCGGG
1101  TAAA    (SEQ ID NO: 35)
```

The mature ActRIIB(K55E)-G1Fc fusion polypeptide (SEQ ID NO: 36) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
   1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDERLHC 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 GQPENNYKTT PPVLDSDGSF
```

301 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
(SEQ ID NO: 36)

The amino acid sequence of unprocessed ActRIIB(F82I)-G1Fc is shown below (SEQ ID NO: 37). The signal sequence and linker sequence are indicated by solid underline, and the F82I substitution is indicated by double underline. The amino acid sequence of SEQ ID NO: 37 may optionally be provided with the lysine removed from the C-terminus.

1 MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDINC 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 NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQK SLSLSPGK (SEQ ID NO: 37)

This ActRIIB(F82I)-G1Fc fusion polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 38):

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 CATCAACTGC 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 CATCCCGGGA GGAGATGACC AAGAACCAGG
851 TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
901 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
951 CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001 ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051 GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG

1101 TAAA (SEQ ID NO: 38)

The mature ActRIIB(F82I)-G1Fc fusion polypeptide (SEQ ID NO: 39) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1 GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51 IELVKKGCWL DDINCYDRQE 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 GQPENNYKTT PPVLDSDGSF
301 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
    (SEQ ID NO: 39)
```

The amino acid sequence of unprocessed ActRIIB(F82K)-G1Fc is shown below (SEQ ID NO: 40). The signal sequence and linker sequence are indicated by solid underline, and the F82K substitution is indicated by double underline. The amino acid sequence of SEQ ID NO: 40 may optionally be provided with the lysine removed from the C-terminus.

```
  1 MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDKNC 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 NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQK SLSLSPGK   (SEQ ID NO: 40)
```

This ActRIIB(F82K)-G1Fc fusion polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 41):

```
  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 CAAGAACTGC 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 CATCCCGGGA GGAGATGACC AAGAACCAGG
 851  TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
 951  CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
1101  TAAA      (SEQ ID NO: 41)
```

The mature ActRIIB(F82K)-G1Fc fusion polypeptide (SEQ ID NO: 42) is as follows and may optionally be provided with the lysine removed from the C-terminus.

```
   1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
  51  IELVKKGCWL DDKNCYDRQE 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 GQPENNYKTT PPVLDSDGSF
 301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
      (SEQ ID NO: 42)
```

Constructs were expressed in COS or CHO cells and purified by filtration and protein A chromatography. In some instances, assays were performed with conditioned medium rather than purified proteins. Purity of samples for reporter gene assays was evaluated by SDS-PAGE and Western blot analysis.

Mutants were tested in binding assays and/or bioassays described below.

Alternatively, similar mutations could be introduced into an ActRIIB extracelluar domain possessing an N-terminal truncation of five amino acids and a C-terminal truncation of three amino acids as shown below (SEQ ID NO: 53). This truncated ActRIIB extracellular domain is denoted ActRIIB(25-131) based on numbering in SEQ ID NO: 2.

```
  25  ETRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
  75  KGCWLDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV
 125  TYEPPPT      (SEQ ID NO: 53)
```

The corresponding background fusion polypeptide, ActRIIB(25-131)-G1Fc, is shown below (SEQ ID NO: 12).

```
  1  ETRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
 51  KGCWLDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV
101  TYEPPPTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
151  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
201  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ
251  VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
301  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK  (SEQ ID NO: 12)
```

Example 3. Activity of Variant ActRIIB-Fc Proteins in a Cell-Based Assay

An A204 cell-based assay was used to compare effects among variant ActRIIB-Fc proteins on signaling by activin A, GDF11, and BMP9. In brief, this assay uses a human A204 rhabdomyosarcoma cell line (ATCC®: HTB-82™) derived from muscle and the reporter vector pGL3(CAGA)12 (Dennler *et al.*, 1998, EMBO 17: 3091-3100) as well as a Renilla reporter plasmid (pRLCMV) to control for transfection efficiency. The CAGA12 motif is present in TGF-β responsive genes (*e.g.*, PAI-1 gene), so this vector is of general use for ligands that can signal through Smad2/3, including activin A, GDF11, and BMP9.

On day 1, A-204 cells were transferred into one or more 48-well plates. On day 2, these cells were transfected with 10 μg pGL3(CAGA)12 or pGL3(CAGA)12(10 μg) + pRLCMV (1 μg) and Fugene. On day 3, ligands diluted in medium containing 0.1% BSA were preincubated with ActRIIB-Fc proteins for 1 hr before addition to cells. Approximately six hour later, the cells were rinsed with PBS and lysed. Cell lysates were analyzed in a luciferase assay to determine the extent of Smad activation.

This assay was used to screen variant ActRIIB-Fc proteins for inhibitory effects on cell signaling by activin A, GDF11, and BMP9. Potencies of homodimeric Fc fusion proteins incorporating amino acid substitutions in the human ActRIIB extracellular domain were compared with that of an Fc fusion protein comprising unmodified human ActRIIB extracellular domain.

Inhibitory Potency of Homodimeric ActRIIB-Fc Constructs			
ActRIIB protein	IC_{50} (ng/mL)		
	Activin A	GDF11	BMP9
Wild-type	8	9	31
A24N	128	99	409
R40A	---	591	1210

E50K	132	180	721
E50P	756	638	~3000
E52A	198	71	359
E52K	762	296	~10000
K55A	15	11	122
K55D	396	365	5500
K55E	19	14	290
K55R	206	318	777
Y60K	---	414	Neg
Y60P	---	544	Neg
K74R	---	45	165
K74Y	---	Neg	Neg
K74A / L79P	---	Neg	Neg
L79K	---	477	Neg
L79P	---	Neg	Neg
L79R	---	234	Neg
D80A	---	Neg	Neg
F82I	11	9	277
F82K	10	15	~5000
F82W	---	276	Neg
F82W / N83A	---	389	~40000
V99E	---	Neg	Neg
V99K	---	Neg	---
Neg Absence of inhibition over concentration range tested --- Not tested			

As shown in the table above, single amino acid substitutions in the ActRIIB extracellular domain can alter the balance between activin A or GDF11 inhibition and BMP9 inhibition in a cell-based reporter gene assay. Compared to a fusion protein containing unmodified ActRIIB extracellular domain, the variants ActRIIB(K55A)-Fc, ActRIIB(K55E)-Fc, ActRIIB(F82I)-Fc, and ActRIIB(F82K)-Fc showed less potent inhibition of BMP9 (increased IC_{50} values) while maintaining essentially undiminished inhibition of activin A and GDF11.

These results indicate that variant ActRIIB-Fc proteins such as ActRIIB(K55A)-Fc, ActRIIB(K55E)-Fc, ActRIIB(F82I)-Fc, and ActRIIB(F82K)-Fc are more selective

antagonists of activin A and GDF11 compared to an Fc fusion protein comprising unmodified ActRIIB extracellular domain. Accordingly, these variants may be more useful than ActRIIB-Fc 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, GDF8, and GDF11 while reducing antagonism of BMP9 and potentially BMP10.

Example 4. Ligand Binding Profiles of Variant ActRIIB-Fc Homodimers

A Biacore™-based binding assay was used to compare ligand binding kinetics of certain variant ActRIIB-Fc proteins screened in Example 3 as well as other variant ActRIIB-Fc proteins not evaluated previously. ActRIIB-Fc proteins to be tested were independently captured onto the system using an anti-Fc antibody. Ligands were then injected and allowed to flow over the captured receptor protein. Results of variant ActRIIB-Fc proteins analyzed at 37°C are shown in **Figure 8**. Compared to Fc-fusion protein comprising unmodified ActRIIB extracellular domain, the variant proteins ActRIIB(K55A)-Fc, ActRIIB(K55E)-Fc, ActRIIB(F82I)-Fc, and ActRIIB(F82K)-Fc exhibited greater reduction in their affinity for BMP9 than for GDF11. Results of additional variant ActRIIB-Fc proteins analyzed at 25°C are shown in **Figure 9**.

These results confirm K55A, K55E, F82I, and F82K as substitutions that reduce ActRIIB binding affinity for BMP9 more than they reduce ActRIIB affinity for activin A or GDF11. Accordingly, these variant ActRIIB-Fc proteins may be more useful than unmodified ActRIIB-Fc protein in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of one or more of activin A, activin B, GDF8, and GDF11 while reducing antagonism of BMP9.

Example 5. Activity of Variant ActRIIB-Fc Homodimers in Mice

Selected variant ActRIIB-G1Fc homodimers were tested in mice to investigate differences in their activity profiles in vivo. Adult wild-type C57BL/6 mice were dosed at 10 mg/kg (i.p.) with ActRIIB(K55A)-Fc, ActRIIB(K55E)-Fc, ActRIIB(F82I)-Fc, ActRIIB(F82K)-Fc, unmodified ActRIIB-Fc, or vehicle twice per week for 4 weeks (n = 8

mice per group). Study endpoints included: body weight, CBC, and total lean mass and total adipose mass as determined by nuclear magnetic resonance (NMR) at baseline and study completion.

Treatment of mice with unmodified ActRIIB-Fc more than tripled the gain in body weight over the course of the study compared to vehicle-treated controls. The increase in body weight caused by ActRIIB(F82I)-Fc (25%) was nearly as large as that caused by unmodified ActRIIB-Fc (29%), while the other variant ActRIIB-Fc proteins produced body weight gains in the range of 12–17% (**Figure 10**). NMR analysis revealed that ActRIIB(F82I)-Fc treatment significantly increased total lean mass and reduced total fat mass compared to vehicle as shown in the table below.

Test Article	Change in lean mass from baseline	Change in fat mass from baseline
Vehicle	– 2.3% ± 0.6%	17.6% ± 5.8%
ActRIIB-G1Fc	3.1% ± 0.7% (P < 0.001 vs vehicle)	– 40.1% ± 5.6% (P = 0.0011 vs vehicle)
ActRIIB(F82I)-G1Fc	1.5% ± 0.7% (P < 0.001 vs vehicle)	– 19.6% ± 6.3% (P < 0.01 vs vehicle)

ActRIIB(F82I)-Fc produced changes in lean mass and fat mass approximately half the magnitude of those produced by ActRIIB-Fc. 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 over the course of treatment with ActRIIB(F82I)-Fc compared to vehicle.

All five of the ActRIIB-Fc fusion proteins evaluated produced significantly higher values for red blood cell parameters (RBC count, hematocrit, and hemoglobin concentration) than did vehicle, and the stimulatory effect of ActRIIB(F82I)-Fc on these parameters exceeded that of unmodified ActRIIB-Fc (**Figure 11**).

Thus, homodimeric Fc-fusion proteins comprising a variant ActRIIB extracellular domain can exert beneficial anabolic effects on red blood cells and skeletal muscle as well as catabolic effects on adipose tissue similar to those of unmodified ActRIIB-Fc homodimer. However, variant ActRIIB-Fc homodimers bind with reduced affinity to BMP9 compared to unmodified ActRIIB-Fc and so will exert diminished inhibition of processes mediated by that ligand, such as angiogenesis. This novel selectivity will be useful, for example, in treating patients in need of stimulatory effects on red blood cells and muscle as well as inhibitory effects on fat, but not in need of altered angiogenesis.

Example 6. Activity of ActRIIB(F82I)-Fc Homodimer in Non-Human Primates

Applicants then investigated whether ActRIIB(F82I)-Fc homodimer alters RBC parameters in non-human primates. Cynomolgus monkeys (*M. fascicularis*) were treated with ActRIIB(F82I)-G1Fc, unmodified ActRIIB-G1Fc, or unmodified ActRIIA-G1Fc at 9 mg/kg (s.c.) on days 1 and 15 of a 29-day study (n = 4 monkeys per group). As shown in **Figure 12**, ActRIIB(F82I)-Fc treatment increased RBC counts compared to ActRIIB-Fc (negative control in primates) by an amount similar to that of ActRIIA-Fc (postitive control). Comparable results were obtained for hemoglobin concentration and hematocrit (data not shown). These data confirm that ActRIIB(F82I)-Fc homodimer possesses activity in vivo different from that of unmodified ActRIIB-Fc homodimer.

Example 7. Generation of an ActRIIB-Fc:ActRIIB(L79E)-Fc Heterodimer

Applicants envision generation of a soluble ActRIIB-Fc:ActRIIB(L79E)-Fc heteromeric complex comprising the extracellular domains of unmodified human ActRIIB and human ActRIIB with a leucine-to-glutamate substitution at position 79, which are each separately fused to an G1Fc domain with a linker positioned between the extracellular domain and the G1Fc domain. The individual constructs are referred to as ActRIIB-Fc fusion polypeptide and ActRIIB(L79E)-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

A methodology for promoting formation of ActRIIB-Fc:ActRIIB(L79E)-Fc heteromeric complexes, as opposed to the ActRIIB-Fc or ActRIIB(L79E)-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(L79E)-Fc and ActRIIB-Fc polypeptide sequences of SEQ ID NOs: 43-45 and 46-48, respectively, one Fc domain can be altered to introduce cationic amino acids at the interaction face, while the other Fc domain can be altered to introduce anionic amino acids at the interaction face. The ActRIIB(L79E)-Fc fusion polypeptide and ActRIIB-Fc fusion polypeptide can each employ the TPA leader (SEQ ID NO: 8).

The ActRIIB(L79E)-Fc polypeptide sequence (SEQ ID NO: 43) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWEDDFNC 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 SLSLSPG   (SEQ ID NO: 43)
```

The leader (signal) sequence and linker are underlined, and the L79E substitution is indicated by double underline. To promote formation of the ActRIIB-Fc:ActRIIB(L79E)-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with acidic amino acids) 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: 43 may optionally be provided with lysine added to the C-terminus.

This ActRIIB(L79E)-Fc fusion protein can be encoded by the following nucleic acid sequence (SEQ ID NO: 44):

```
  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  GGGAAGATGA 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 CATCCCGGGA GGAGATGACC AAGAACCAGG
 851  TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACGACA CCACGCCTCC
 951  CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCGAC CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
1101  T       (SEQ ID NO: 44)
```

The mature ActRIIB(L79E)-Fc fusion polypeptide (SEQ ID NO: 45) is as follows, and may optionally be provided with lysine added to the C-terminus.

```
   1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
  51  IELVKKGCWE 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 PG
      (SEQ ID NO: 45)
```

The complementary form of ActRIIB-Fc fusion polypeptide (SEQ ID NO: 46) 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 TLPPSRKEMT KNQVSLTCLV KGFYPSDIAV
 301  EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
 351  EALHNHYTQK SLSLSPGK   (SEQ ID NO: 46)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB(L79E)-Fc fusion polypeptide of SEQ ID NOs: 43 and 45 above, two amino acid substitutions (replacing a glutamate and an aspartate with lysines) 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: 46 may optionally be provided with lysine removed from the C-terminus.

This ActRIIB-Fc fusion protein can be encoded by the following nucleic acid (SEQ ID NO: 47):

```
   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: 47)
```

The mature ActRIIB-Fc fusion protein sequence (SEQ ID NO: 48) 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: 48)
```

The ActRIIB(L79E)-Fc and ActRIIB-Fc polypeptides of SEQ ID NO: 45 and SEQ ID NO: 48, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric protein complex comprising ActRIIB-Fc:ActRIIB(L79E)-Fc.

In another 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 as illustrated in the ActRIIB(L79E)-Fc and ActRIIB-Fc polypeptide sequences of SEQ ID NOs: 49-50 and 51-52, respectively. The ActRIIB(L79E)-Fc fusion polypeptide and ActRIIB-Fc fusion polypeptide can each employ the TPA leader (SEQ ID NO: 8).

The ActRIIB(L79E)-Fc polypeptide sequence (SEQ ID NO: 49) is shown below:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWEDDFNC 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 SLSLSPG   (SEQ ID NO: 49)
```

The signal sequence and linker sequence are underlined, and the L79E substitution is indicated by double underline. To promote formation of the ActRIIB-Fc:ActRIIB(L79E)-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: 49 may optionally be provided with lysine added to the C-terminus.

The mature ActRIIB(L79E)-Fc fusion polypeptide (SEQ ID NO: 50) is as follows:

```
  1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51  IELVKKGCWE 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 PG
     (SEQ ID NO: 50)
```

The complementary form of ActRIIB-Fc fusion polypeptide (SEQ ID NO: 51) is as follows and may optionally be provided with lysine removed from the C-terminus.

```
  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: 51)
```

The leader sequence and linker are underlined. To guide heterodimer formation with the ActRIIB(L79E)-Fc fusion polypeptide of SEQ ID NOs: 49-50 above, four amino acid substitutions (replacement of tyrosine with cysteine, threonine with serine, leucine with alanine, and tyrosine with valine) 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: 51 may optionally be provided with lysine removed from the C-terminus.

The mature ActRIIB-Fc fusion protein sequence 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 EPQVCTLPPS
251  REEMTKNQVS LSCAVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301  FLVSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
     (SEQ ID NO: 52)
```

The ActRIIB(L79E)-Fc and ActRIIB-Fc polypeptides of SEQ ID NO: 50 and SEQ ID NO: 52, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric protein complex comprising ActRIIB-Fc:ActRIIB(L79E)-Fc.

Purification of various ActRIIB-Fc:ActRIIB(L79E)-Fc complexes can 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, cation exchange chromatography, multimodal chromatography (e.g., with resin containing both electrostatic and hydrophobic ligands), and epitope-based affinity chromatography (e.g., with an antibody

or functionally equivalent ligand directed against an epitope of ActRIIB). The purification can be completed with viral filtration and buffer exchange.

Example 8. Ligand Binding Profile of ActRIIB-Fc:ActRIIB(L79E)-Fc Heteromer

A Biacore™-based binding assay was used to compare the ligand binding kinetics of an ActRIIB-Fc:ActRIIB(L79E)-Fc heterodimer with those of unmodified ActRIIB-Fc homodimer. Fusion proteins were captured onto the system using an anti-Fc antibody. Ligands were then injected and allowed to flow over the captured receptor protein at 37°C. 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 of ActRIIB-Fc:ActRIIB(L79E)-Fc heterodimer compared to ActRII-Fc homodimer at 37°C						
Ligand	ActRIIB-Fc homodimer			ActRIIB-Fc:ActRIIB(L79E)-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	7.4×10^6	1.9×10^{-4}	25	8.8×10^6	1.5×10^{-3}	170
Activin B	8.1×10^6	6.6×10^{-5}	8	8.3×10^6	2.1×10^{-4}	25
GDF3	1.4×10^6	2.2×10^{-3}	1500	5.8×10^5	5.9×10^{-3}	10000
GDF8	3.8×10^6	2.6×10^{-4}	70	3.4×10^6	5.0×10^{-4}	150
GDF11	4.1×10^7	1.7×10^{-4}	4	4.0×10^7	3.6×10^{-4}	9
BMP6	1.3×10^8	7.4×10^{-3}	56	3.3×10^8	1.8×10^{-2}	56
BMP9	5.0×10^6	1.3×10^{-3}	250	Transient*		>2800
BMP10	5.1×10^7	2.0×10^{-4}	4	4.8×10^7	2.0×10^{-3}	42
* Indeterminate due to transient nature of interaction						

In this example, a single amino acid substitution in one of two ActRIIB polypeptide chains altered ligand binding selectivity of the Fc-fusion protein relative to unmodifed ActRIIB-Fc homodimer. Compared to ActRIIB-Fc homodimer, the ActRIIB(L79E)-Fc heterodimer largely retained high-affinity binding to activin B, GDF8, GDF11, and BMP6 but exhibited approximately ten-fold faster off-rates for activin A and BMP10 and an even greater reduction in the strength of binding to BMP9. Accordingly, a variant ActRIIB-Fc heteromer may be more useful than unmodified 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 B, GDF8, GDF11, and BMP6, while reducing antagonism of activin A, BMP9, or BMP10.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

While specific embodiments of the subject matter have been discussed, the above specification is illustrative and not restrictive. Many variations will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

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: 196)

ACGATCCCACCGCACGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACAACGG
TGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACC
AGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGTCTGT
GTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTGCCATGACCCCAA
GCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAA
AAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCAATGACAAC
ATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAA
(SEQ ID NO: 197)

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: 202)

ACGATCCCACCGCACGTTCAGAAGTCGGATGTGGAAATGGAGGCCCAGAAAGATGAAATCAT
CTGCCCCAGCTGTAATAGGACTGCCCATCCACTGAGACATATTAATAACGACATGATAGTCA
CTGACAACAACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCC
ACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCC
ACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTT
GCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGC
ATTATGAAGGAAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGA
GTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAG
TCATATTTCAA (SEQ ID NO: 203).

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGTCAGCACTGCGGCT
GCTTCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGGTGAGAGTAGA
ATCTCTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGG
GACATAAATCTTGTAAAACAAGGATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTA
GTAACTACCACTCCTCCCTCAATTCAGAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTC
AACTTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
ATAATCATTGCTTTGGCATCAGTCTCTGTATTAGCTGTTTTGATAGTTGCCTTATGCTTTGGATACAGAATGTTG
ACAGGAGACCGTAAACAAGGTCTTCACAGTATGAACATGATGGAGGCAGCAGCATCCGAACCCTCTCTTGATCTA
GATAATCTGAAACTGTTGGAGCTGATTGGCCGAGGTCGATATGGAGCAGTATATAAAGGCTCCTTGGATGAGCGT
CCAGTTGCTGTAAAAGTGTTTTCCTTTGCAAACCGTCAGAATTTTATCAACGAAAAGAACATTTACAGAGTGCCT
TTGATGGAACATGACAACATTGCCCGCTTTATAGTTGGAGATGAGAGAGTCACTGCAGATGGACGCATGGAATAT
TTGCTTGTGATGGAGTACTATCCCAATGGATCTTTATGCAAGTATTTAAGTCTCCACACAAGTGACTGGGTAAGC
TCTTGCCGTCTTGCTCATTCTGTTACTAGAGGACTGGCTTATCTTCACACAGAATTACCACGAGGAGATCATTAT
AAACCTGCAATTTCCCATCGAGATTTAAACAGCAGAAATGTCCTAGTGAAAAATGATGGAACCTGTGTTATTAGT
GACTTTGGACTGTCCATGAGGCTGACTGGAAATAGACTGGTGCGCCCAGGGGAGGAAGATAATGCAGCCATAAGC
GAGGTTGGCACTATCAGATATATGGCACCAGAAGTGCTAGAAGGAGCTGTGAACTTGAGGGACTGTGAATCAGCT
TTGAAACAAGTAGACATGTATGCTCTTGGACTAATCTATTGGGAGATATTTATGAGATGTACAGACCTCTTCCCA
GGGGAATCCGTACCAGAGTACCAGATGGCTTTTCAGACAGAGGTTGGAAACCATCCCACTTTTGAGGATATGCAG
GTTCTCGTGTCTAGGGAAAAACAGAGACCCAAGTTCCCAGAAGCCTGGAAAGAAAATAGCCTGGCAGTGAGGTCA
CTCAAGGAGACAATCGAAGACTGTTGGGACCAGGATGCAGAGGCTCGGCTTACTGCACAGTGTGCTGAGGAAAGG
ATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAACAGTCAATCCAATGTCTACTGCTATG
CAGAATGAACGCAACCTGTCACATAATAGGCGTGTGCCAAAAATTGGTCCTTATCCAGATTATTCTTCCTCCTCA
TACATTGAAGACTCTATCCATCATACTGACAGCATCGTGAAGAATATTTCCTCTGAGCATTCTATGTCCAGCACA
CCTTTGACTATAGGGGAAAAAAACCGAAATTCAATTAACTATGAACGACAGCAAGCACAAGCTCGAATCCCCAGC
CCTGAAACAAGTGTCACCAGCCTCTCCACCAACACAACAACCACAAACACCACAGGACTCACGCCAAGTACTGGC
ATGACTACTATATCTGAGATGCCATACCCAGATGAAACAAATCTGCATACCACAAATGTTGCACAGTCAATTGGG
CCAACCCCTGTCTGCTTACAGCTGACAGAAGAAGACTTGGAAACCAACAAGCTAGACCCAAAAGAAGTTGATAAG
AACCTCAAGGAAAGCTCTGATGAGAATCTCATGGAGCACTCTCTTAAACAGTTCAGTGGCCCAGACCCACTGAGC
AGTACTAGTTCTAGCTTGCTTTACCCACTCATAAAACTTGCAGTAGAAGCAACTGGACAGCAGGACTTCACACAG
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CTTCCCAAGAGACCTACTAGTTTGCCTTTGAACACCAAAAATTCAACAAAAGAGCCCCGGCTAAAATTTGGCAGC
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GTGGTGACAGTCACCATGAATGGTGTGGCAGGTAGAAACCACAGTGTTAACTCCCATGCTGCCACAACCCAATAT
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CAAGCTGGCCATGATGAAGGTGTTCTGGATCGTCTTGTGGACAGGAGGGAACGGCCACTAGAAGGTGGCCGAACT
AATTCCAATAACAACAACAGCAATCCATGTTCAGAACAAGATGTTCTTGCACAGGGTGTTCCAAGCACAGCAGCA
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GGCAGCAGTATACAGATAGGTGAGTCAACACAAGATGGCAAATCAGGATCAGGTGAAAAGATCAAGAAACGTGTG
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AACAATAATGGCAGTAACAGGGCAGTTCATTCCAAATCCAGCACTGCTGTTTACCTTGCAGAAGGAGGCACTGCT
ACAACCATGGTGTCTAAAGATATAGGAATGAACTGTCTG (SEQ ID NO: 205)

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGGTGAGAGTAGAATC
TCTCATGAAAATGGGACAATATTATGCTCGAAAGGTAGCACCTGCTATGGCCTTTGGGAGAAATCAAAAGGGGAC
ATAAATCTTGTAAAACAAGGATGTTGGTCTCACATTGGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTA
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TTTACTGAGAATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
(SEQ ID NO: 206)

ATGACTTCCTCGCTGCAGCGGCCCTGGCGGGTGCCCTGGCTACCATGGACCATCCTGCTGGT
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GCTGAGGAAAGGATGGCTGAACTTATGATGATTTGGGAAAGAAACAAATCTGTGAGCCCAAC
AGTCAATCCAATGTCTACTGCTATGCAGAATGAACGTAGG (SEQ ID NO: 207)

TCGCAGAATCAAGAACGGCTATGTGCGTTTAAAGATCCGTATCAGCAAGACCTTGGGATAGG
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GCCTTTGGGAGAAATCAAAAGGGGACATAAATCTTGTAAAACAAGGATGTTGGTCTCACATT
GGAGATCCCCAAGAGTGTCACTATGAAGAATGTGTAGTAACTACCACTCCTCCCTCAATTCA
GAATGGAACATACCGTTTCTGCTGTTGTAGCACAGATTTATGTAATGTCAACTTTACTGAGA
ATTTTCCACCTCCTGACACAACACCACTCAGTCCACCTCATTCATTTAACCGAGATGAGACA
(SEQ ID NO: 208)

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCA**CCCCCAAACAGGCGAACCTGTGTG**
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TCCCTGCGGATGGCACTGTCCCTGGCCCAGGGCCTGGCATTTCTCCATGAGGAGCGCTGGCAGAATGGCCAATAT
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GCTGCCATCATGGAAGCTGGCACCCAGAGGTACATGGCACCAGAGCTCTTGGACAAGACTCTGGACCTACAGGAT
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ID NO: 209)

CCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGAC
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GCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGGATGC
CGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACCC
CAGCCCTGGCTCCACTCTCTTCACCTGCTCCTGTGGCACTGACTTCTGCAATGCCAATTACA
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GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 210)

ATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAGCTGTGGAAGCACCCCCAAACAGGCGAACCTGTGTG
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CTGATGGGC (SEQ ID NO: 211)

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GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 212)

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GGTGAGTCCATCTGGATGGCACTG (SEQ ID NO: 214)

ATGACCTTGGGCTCCCCCAGGAAAGGCCTTCTGATGCTGCTGATGGCCTTGGTGACCCAGGGA**GACCCTGTGAAG**
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ATGGAAGATGAGAAGCCCAAGGTCAACCCCAAACTCTACATGTGTGTGTGTGAAGGTCTCTC
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(SEQ ID NO: 218)

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
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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
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1081 CGAGACCTAA AGAGCAAAAA CATCCTCATC AAGAAAAATG GGAGTTGCTG CATTGCTGAC
1141 CTGGGCCTTG CTGTTAAATT CAACAGTGAC ACAAATGAAG TTGATGTGCC CTTGAATACC
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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: 219)

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 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: 220)
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ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGC
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GAAGCTGCGTCCCAACATCCCCAACTGGTGGCAGAGTTATGAGGCACTGCGGGT
GATGGGGAAGATGATGCGAGAGTGTTGGTATGCCAACGGCGCAGCCCGCCTGAC
GGCCCTGCGCATCAAGAAGACCCTCTCCCAGCTCAGCGTGCAGGAAGACGTGAA
GATC (SEQ ID NO: 221)

TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTA
CACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACC
ATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTACTGCCTG
AGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGATCGACTT
GAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAG
(SEQ ID NO: 222)

ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCTTCCCCCTTGTTGTCCTCCTGCTCGCCGGCAGCGGCGGG**TCCGGG**
CCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTACACGTGTGAGACAGATGGG
GCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACCATGTGCGCACCTGCATCCCCAAAGTGGAGCTG
GTCCCTGCCGGGAAGCCCTTCTACTGCCTGAGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTAC
TGCAACAGGATCGACTTGAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTG
GAGCTGGTAGGCATCATCGCCGGCCCGGTGTTCCTCCTGTTCCTCATCATCATCATTGTTTTCCTTGTCATTAAC
TATCATCAGCGTGTCTATCACAACCGCCAGAGACTGGACATGGAAGATCCCTCATGTGAGATGTGTCTCTCCAAA
GACAAGACGCTCCAGGATCTTGTCTACGATCTCTCCACCTCAGGGTCTGGCTCAGGGTTACCCCTCTTTGTCCAG
CGCACAGTGGCCCGAACCATCGTTTTACAAGAGATTATTGGCAAGGGTCGGTTTGGGGAAGTATGGCGGGGCCGC
TGGAGGGGTGGTGATGTGGCTGTGAAAATATTCTCTTCTCGTGAAGAACGGTCTTGGTTCAGGGAAGCAGAGATA
TACCAGACGGTCATGCTGCGCCATGAAAACATCCTTGGATTTATTGCTGCTGACAATAAAGCAGACTGCTCATTC
CTCACATTGCCATGGGAAGTTGTAATGGTCTCTGCTGCCCCCAAGCTGAGGAGCCTTAGACTCCAATACAAGGGA
GGAAGGGGAAGAGCAAGATTTTTATTCCCACTGAATAATGGCACCTGGACACAGCTGTGGCTTGTTTCTGACTAT
CATGAGCACGGGTCCCTGTTTGATTATCTGAACCGGTACACAGTGACAATTGAGGGGATGATTAAGCTGGCCTTG
TCTGCTGCTAGTGGGCTGGCACACCTGCACATGGAGATCGTGGGCACCCAAGGGAAGCCTGGAATTGCTCATCGA
GACTTAAAGTCAAAGAACATTCTGGTGAAGAAAAATGGCATGTGTGCCATAGCAGACCTGGGCCTGGCTGTCCGT
CATGATGCAGTCACTGACACCATTGACATTGCCCCGAATCAGAGGGTGGGGACCAAACGATACATGGCCCCTGAA
GTACTTGATGAAACCATTAATATGAAACACTTTGACTCCTTTAAATGTGCTGATATTTATGCCCTCGGGCTTGTA
TATTGGGAGATTGCTCGAAGATGCAATTCTGGAGGAGTCCATGAAGAATATCAGCTGCCATATTACGACTTAGTG
CCCTCTGACCCTTCCATTGAGGAAATGCGAAAGGTTGTATGTGATCAGAAGCTGCGTCCCAACATCCCCAACTGG
TGGCAGAGTTATGAGGCACTGCGGGTGATGGGGAAGATGATGCGAGAGTGTTGGTATGCCAACGGCGCAGCCCGC
CTGACGGCCCTGCGCATCAAGAAGACCCTCTCCCAGCTCAGCGTGCAGGAAGACGTGAAGATC (SEQ ID NO: 223)

TCCGGGCCCCGGGGGGTCCAGGCTCTGCTGTGTGCGTGCACCAGCTGCCTCCAGGCCAACTA
CACGTGTGAGACAGATGGGGCCTGCATGGTTTCCATTTTCAATCTGGATGGGATGGAGCACC
ATGTGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTTCTACTGCCTG
AGCTCGGAGGACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGATCGACTT
GAGGGTGCCCAGTGGTCACCTCAAGGAGCCTGAGCACCCGTCCATGTGGGGCCCGGTGGAG
(SEQ ID NO: 224)

ATGGAGGCGGCGGTCGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGCGGCGGCGGCG**GCG**
GCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAATTTTACTTGTGTGACA
GATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTATACACAACAGCATGTGTATAGCTGAAATT
GACTTAATTCCTCGAGATAGGCCGTTTGTATGTGCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGC
TGCAATCAGGACCATTGCAATAAAATAGAACTTCCAACTACTGTAAAGTCATCACCTGGCCTTGGTCCTGTGGAA
CTGGCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACTCATGTTGATGGTCTATATCTGCCACAAC
CGCACTGTCATTCACCATCGAGTGCCAAATGAAGAGGACCCTTCATTAGATCGCCCTTTTATTTCAGAGGGTACT
ACGTTGAAAGACTTAATTTATGATATGACAACGTCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAACA
ATTGCGAGAACTATTGTGTTACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGG
GGAGAAGAAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTCGTGGTTCCGTGAGGCAGAGATTTATCAA
ACTGTAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGCAGACAATAAAGACAATGGTACTTGGACTCAG
CTCTGGTTGGTGTCAGATTATCATGAGCATGGATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAA
GGAATGATAAAACTTGCTCTGTCCACGGCGAGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGA
AAGCCAGCCATTGCTCATAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACTTGCTGTATTGCA
GACTTAGGACTGGCAGTAAGACATGATTCAGCCACAGATACCATTGATATTGCTCCAAACCACAGAGTGGGAACA
AAAAGGTACATGGCCCCTGAAGTTCTCGATGATTCCATAAATATGAAACATTTTGAATCCTTCAAACGTGCTGAC
ATCTATGCAATGGGCTTAGTATTCTGGGAAATTGCTCGACGATGTTCCATTGGTGGAATTCATGAAGATTACCAA
CTGCCTTATTATGATCTTGTACCTTCTGACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTA
AGGCCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGAGAGAATGTTGG
TATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATTAAGAAAACATTATCGCAACTCAGTCAACAGGAAGGC
ATCAAAATG (SEQ ID NO: 225)

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
TTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTA
TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
GCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
TAAAATAGAACTTCCAACTACTGTAAAGTCATCACCTGGCCTTGGTCCTGTGGAACTG
(SEQ ID NO: 226)

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: 227)

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
TTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTCACAGAGACCACAGACAAAGTTA
TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
GCACCCTCTTCAAAAACTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
TAAAATAGAACTTCCAACTACTGGCCCTTTTTCAGTAAAGTCATCACCTGGCCTTGGTCCTG
TGGAACTG (SEQ ID NO: 228)

ATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACC**AAGAAAGAGGATGGTGAGAGTACAGCCCCCACCCCC**
CGTCCAAAGGTCTTGCGTTGTAAATGCCACCACCATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGAC
GGATATTGTTTCACGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAA
GGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAAGGAAC
GAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTTGATGGACCTATACACCAC
AGGGCTTTACTTATATCTGTGACTGTCTGTAGTTTGCTCTTGGTCCTTATCATATTATTTTGTTACTTCCGGTAT
AAAAGACAAGAAACCAGACCTCGATACAGCATTGGGTTAGAACAGGATGAAACTTACATTCCTCCTGGAGAATCC
CTGAGAGACTTAATTGAGCAGTCTCAGAGCTCAGGAAGTGGATCAGGCCTCCCTCTGCTGGTCCAAAGGACTATA
GCTAAGCAGATTCAGATGGTGAAACAGATTGGAAAAGGTCGCTATGGGGAAGTTTGGATGGGAAAGTGGCGTGGC
GAAAAGGTAGCTGTGAAAGTGTTCTTCACCACAGAGGAAGCCAGCTGGTTCAGAGAGACAGAAATATATCAGACA
GTGTTGATGAGGCATGAAAACATTTTGGGTTTCATTGCTGCAGATATCAAAGGGACAGGGTCCTGGACCCAGTTG
TACCTAATCACAGACTATCATGAAAATGGTTCCCTTTATGATTATCTGAAGTCCACCACCCTAGACGCTAAATCA
ATGCTGAAGTTAGCCTACTCTTCTGTCAGTGGCTTATGTCATTTACACACAGAAATCTTTAGTACTCAAGGCAAA
CCAGCAATTGCCCATCGAGATCTGAAAAGTAAAAACATTCTGGTGAAGAAAAATGGAACTTGCTGTATTGCTGAC
CTGGGCCTGGCTGTTAAATTTATTAGTGATACAAATGAAGTTGACATACCACCTAACACTCGAGTTGGCACCAAA
CGCTATATGCCTCCAGAAGTGTTGGACGAGAGCTTGAACAGAAATCACTTCCAGTCTTACATCATGGCTGACATG
TATAGTTTTGGCCTCATCCTTTGGGAGGTTGCTAGGAGATGTGTATCAGGAGGTATAGTGGAAGAATACCAGCTT
CCTTATCATGACCTAGTGCCCAGTGACCCCTCTTATGAGGACATGAGGGAGATTGTGTGCATCAAGAAGTTACGC
CCCTCATTCCCAAACCGGTGGAGCAGTGATGAGTGTCTAAGGCAGATGGGAAAACTCATGACAGAATGCTGGGCT
CACAATCCTGCATCAAGGCTGACAGCCCTGCGGGTTAAGAAAACACTTGCCAAAATGTCAGAGTCCCAGGACATT
AAACTC (SEQ ID NO: 229)

AAGAAAGAGGATGGTGAGAGTACAGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATG
CCACCACCATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCA
CGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAA
GGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTG
CACAGAAAGGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAG
ATTTTGTTGATGGACCTATACACCACAGG (SEQ ID NO: 230)

ATGGGTTGGCTGGAAGAACTAAACTGGCAGCTTCACATTTTCTTGCTCATTCTTCTCTCTATGCACACAAGGGCA
AACTTCCTTGATAACATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACCAAGAAAGAGGATGGTGAGAGT
ACAGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATGCCACCACCATTGTCCAGAAGACTCAGTCAACAAT
ATTTGCAGCACAGACGGATATTGTTTCACGATGATAGAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGT
TGCCTAGGACTAGAAGGCTCAGATTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGC
TGCACAGAAAGGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTTGAT
GGACCTATACACCACAGGGCTTTACTTATATCTGTGACTGTCTGTAGTTTGCTCTTGGTCCTTATCATATTATTT
TGTTACTTCCGGTATAAAAGACAAGAAACCAGACCTCGATACAGCATTGGGTTAGAACAGGATGAAACTTACATT
CCTCCTGGAGAATCCCTGAGAGACTTAATTGAGCAGTCTCAGAGCTCAGGAAGTGGATCAGGCCTCCCTCTGCTG
GTCCAAAGGACTATAGCTAAGCAGATTCAGATGGTGAAACAGATTGGAAAAGGTCGCTATGGGGAAGTTTGGATG
GGAAAGTGGCGTGGCGAAAAGGTAGCTGTGAAAGTGTTCTTCACCACAGAGGAAGCCAGCTGGTTCAGAGAGACA
GAAATATATCAGACAGTGTTGATGAGGCATGAAAACATTTTGGGTTTCATTGCTGCAGATATCAAAGGGACAGGG
TCCTGGACCCAGTTGTACCTAATCACAGACTATCATGAAAATGGTTCCCTTTATGATTATCTGAAGTCCACCACC
CTAGACGCTAAATCAATGCTGAAGTTAGCCTACTCTTCTGTCAGTGGCTTATGTCATTTACACACAGAAATCTTT
AGTACTCAAGGCAAACCAGCAATTGCCCATCGAGATCTGAAAAGTAAAAACATTCTGGTGAAGAAAAATGGAACT
TGCTGTATTGCTGACCTGGGCCTGGCTGTTAAATTTATTAGTGATACAAATGAAGTTGACATACCACCTAACACT
CGAGTTGGCACCAAACGCTATATGCCTCCAGAAGTGTTGGACGAGAGCTTGAACAGAAATCACTTCCAGTCTTAC
ATCATGGCTGACATGTATAGTTTTGGCCTCATCCTTTGGGAGGTTGCTAGGAGATGTGTATCAGGAGGTATAGTG
GAAGAATACCAGCTTCCTTATCATGACCTAGTGCCCAGTGACCCCTCTTATGAGGACATGAGGGAGATTGTGTGC
ATCAAGAAGTTACGCCCCTCATTCCCAAACCGGTGGAGCAGTGATGAGTGTCTAAGGCAGATGGGAAAACTCATG
ACAGAATGCTGGGCTCACAATCCTGCATCAAGGCTGACAGCCCTGCGGGTTAAGAAAACACTTGCCAAAATGTCA
GAGTCCCAGGACATTAAACTC (SEQ ID NO: 231)

AACTTCCTTGATAACATGCTTTTGCGAAGTGCAGGAAAATTAAATGTGGGCACCAAGAAAGA
GGATGGTGAGAGTACAGCCCCCACCCCCCGTCCAAAGGTCTTGCGTTGTAAATGCCACCACC
ATTGTCCAGAAGACTCAGTCAACAATATTTGCAGCACAGACGGATATTGTTTCACGATGATA
GAAGAGGATGACTCTGGGTTGCCTGTGGTCACTTCTGGTTGCCTAGGACTAGAAGGCTCAGA
TTTTCAGTGTCGGGACACTCCCATTCCTCATCAAAGAAGATCAATTGAATGCTGCACAGAAA
GGAACGAATGTAATAAAGACCTACACCCTACACTGCCTCCATTGAAAAACAGAGATTTTGTT
GATGGACCTATACACCACAGG (SEQ ID NO: 232)

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCC**GAGCTCTCGCCAGGA**
CTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCATGTTGGGCATCAGTC
ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGT
CATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCA
ACAGCATCACCAAATGCCCCAAAACTTGGACCCATGGAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTCCTG
TCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTGCTCCTACAGGAAGAAAAAGAGACCAAAT
GTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTGGAAAAACTCTGAAAGATCTGATTTATGATGTGACC
GCCTCTGGATCTGGCTCTGGTCTACCTCTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATA
GTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCC
TCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTT
GGTTTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAG
GGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCT
AGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAA
TCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCA
ATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGAT
GATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAA
ATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGAT
CCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGT
TGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCT
CTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 233)

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCA
AACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAAT
CCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATC
ACCAAATGCCCCAAAACTTGGACCCATGGAG (SEQ ID NO: 234)

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGC
TCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTT
GCAACAACATAACACTGCACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATG
GAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTCCTGTCCATAGCTGCGATGCTGACAGT
ATGGGCATGCCAGGGTCGACAGTGCTCCTACAGGAAGAAAAAGAGACCAAATGTGGAGGAAC
CACTCTCTGAGTGCAATCTGGTAAATGCTGGAAAAACTCTGAAAGATCTGATTTATGATGTG
ACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTGGTTCAAAGGACAATTGCAAGGACGAT
TGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTG
GGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCA
GAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAA
CAAAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCT
TATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCA
ATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTAT
TGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAG
CGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAAT
CCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAA
TATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAG
CCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTG
CCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAG
TATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGT
GTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAA
CTTTGTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 235)

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGC
TCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTT
GCAACAACATAACACTGCACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATG
GAG (SEQ ID NO: 236)

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGA
GCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAA
CAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCC
TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAA
AACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGGTCTAC
CTCTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGT
AGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTC
CTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGAC
ATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAACTT
TGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGT
GACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATA
TGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAAT
ATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGA
TTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGG
CTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGAC
ATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGT
TGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGA
GAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAA
GCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCT
AACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC

(SEQ ID NO: 237)

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCA
AACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAAT
CCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGGTCT
ACCTCTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAG
GTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTC
TCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCG
ACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAAC
TTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATA
GTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCA
TATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGA
ATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCAT
GATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATAT
GGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTG
ACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATT
GTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAAT
GAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTG
AAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGC
CTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGC
C (SEQ ID NO: 238)

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGA
GCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAA
CAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCC
TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAA
AACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGATAATG
GAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTAT
TTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGG
TCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAG
ACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGG
TTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGG
AACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGT
CCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGT
TCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCC
CTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACC
AGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCC
AACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAA
AGAAGACTGCAAAGCCTAA (SEQ ID NO: 239)

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCA
AACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAAT
CCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGATAA
TGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACT
ATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGT
GGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCG
AGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAG
GGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTG
GGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGA
GTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGT
GTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGAT
CCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAA
CCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATG
CCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTC
AAAGAAGACTGCAAAGCCTAA (SEQ ID NO: 240)

```
   1 ATGGGAGCTG CTGCAAAGTT GGCGTTTGCC GTCTTTCTTA TCTCCTGTTC
  51 TTCAGGTGCT ATACTTGGTA GATCAGAAAC TCAGGAGTGT CTTTTCTTTA
 101 ATGCTAATTG GGAAAAAGAC AGAACCAATC AAACTGGTGT TGAACCGTGT
 151 TATGGTGACA AAGATAAACG GCGGCATTGT TTTGCTACCT GGAAGAATAT
 201 TTCTGGTTCC ATTGAAATAG TGAAACAAGG TTGTTGGCTG GATGATATCA
 251 ACTGCTATGA CAGGACTGAT TGTGTAGAAA AAAAAGACAG CCCTGAAGTA
 301 TATTTTTGTT GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATTT
 351 TCCGGAGATG GAAGTCACAC AGCCCACTTC AAATCCAGTT ACACCTAAGC
 401 CACCCTATTA CAACATCCTG CTCTATTCCT TGGTGCCACT TATGTTAATT
 451 GCGGGGATTG TCATTTGTGC ATTTTGGGTG TACAGGCATC ACAAGATGGC
 501 CTACCCTCCT GTACTTGTTC CAACTCAAGA CCCAGGACCA CCCCCACCTT
 551 CTCCATTACT AGGTTTGAAA CCACTGCAGT TATTAGAAGT GAAAGCAAGG
 601 GGAAGATTTG GTTGTGTCTG GAAAGCCCAG TTGCTTAACG AATATGTGGC
 651 TGTCAAAATA TTTCCAATAC AGGACAAACA GTCATGGCAA AATGAATACG
 701 AAGTCTACAG TTTGCCTGGA ATGAAGCATG AGAACATATT ACAGTTCATT
 751 GGTGCAGAAA AACGAGGCAC CAGTGTTGAT GTGGATCTTT GGCTGATCAC
 801 AGCATTTCAT GAAAAGGGTT CACTATCAGA CTTTCTTAAG GCTAATGTGG
 851 TCTCTTGGAA TGAACTGTGT CATATTGCAG AAACCATGGC TAGAGGATTG
 901 GCATATTTAC ATGAGGATAT ACCTGGCCTA AAAGATGGCC ACAAACCTGC
 951 CATATCTCAC AGGGACATCA AAAGTAAAAA TGTGCTGTTG AAAAACAACC
1001 TGACAGCTTG CATTGCTGAC TTTGGGTTGG CCTTAAAATT TGAGGCTGGC
1051 AAGTCTGCAG GCGATACCCA TGGACAGGTT GGTACCCGGA GGTACATGGC
1101 TCCAGAGGTA TTAGAGGGTG CTATAAACTT CCAAAGGGAT GCATTTTTGA
1151 GGATAGATAT GTATGCCATG GGATTAGTCC TATGGGAACT GGCTTCTCGC
1201 TGTACTGCTG CAGATGGACC TGTAGATGAA TACATGTTGC CATTTGAGGA
1251 GGAAATTGGC CAGCATCCAT CTCTTGAAGA CATGCAGGAA GTTGTTGTGC
1301 ATAAAAAAAA GAGGCCTGTT TTAAGAGATT ATTGGCAGAA ACATGCTGGA
1351 ATGGCAATGC TCTGTGAAAC CATTGAAGAA TGTTGGGATC ACGACGCAGA
1401 AGCCAGGTTA TCAGCTGGAT GTGTAGGTGA AAGAATTACC CAGATGCAGA
1451 GACTAACAAA TATTATTACC ACAGAGGACA TTGTAACAGT GGTCACAATG
1501 GTGACAAATG TTGACTTTCC TCCCAAAGAA TCTAGTCTA
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(SEQ ID NO: 241)

```
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: 242)

Claims:

1. A variant ActRIIB polypeptide comprising an amino acid sequence that is at least 85% identical to an amino acid sequence that begins at any one of amino acids 20-29 of SEQ ID NO: 2 and ends at any one of amino acids 109-134 of SEQ ID NO: 2, and wherein the polypeptide comprises a lysine at the position corresponding to position 82 of SEQ ID NO: 2.

2. The variant ActRIIB polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to amino acids 29-109 of SEQ ID NO: 2.

3. The variant ActRIIB polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to amino acids 25-131 of SEQ ID NO: 2.

4. The variant ActRIIB polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to amino acids 20-134 of SEQ ID NO: 2.

5. The variant ActRIIB polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 53.

6. The variant ActRIIB polypeptide of any one of claims 1-5, wherein the polypeptide is a fusion protein further comprising an Fc polypeptide domain.

7. The variant ActRIIB polypeptide of claim 6, wherein the fusion protein further comprises a linker domain positioned between the ActRIIB polypeptide and the Fc polypeptide domain.

8. The variant ActRIIB polypeptide of claim 7, wherein the linker domain is selected from: TGGG, TGGGG, SGGGG, GGGGS, GGG, GGGG, SGGG, and GGGGS.

9. The variant ActRIIB polypeptide of any one of claims 1-8, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NOs: 5 or 6.

10. The variant ActRIIB polypeptide of any one of claims 1-8, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 12.

11. The variant ActRIIB polypeptide of any one of claims 1-8, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 40.

12. The variant ActRIIB polypeptide of any one of claims 1-8, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 42.

13. The variant ActRIIB polypeptide of any one of claims 1-12, wherein the polypeptide is encoded by a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 41.

14. The variant ActRIIB polypeptide of any one of claims 1-13, wherein the ActRIIB polypeptide is a homodimer or a heterodimer protein.

15. The variant ActRIIB polypeptide of claim 14, wherein the ActRIIB polypeptide is a heterodimer protein, and wherein the heterodimer protein comprises a second ActRIIB polypeptide, wherein the first ActRIIB polypeptide comprises a different amino acid sequence compared to the second ActRIIB polypeptide.

16. The variant ActRIIB polypeptide of claim 15, wherein the second ActRIIB polypeptide is a wild-type ActRIIB extracellular domain polypeptide.

17. The variant ActRIIB polypeptide of any one of claims 1-16, wherein the first ActRIIB polypeptide and/or second ActRIIB polypeptide is glycosylated and has a glycosylation pattern obtainable from of the polypeptide in a CHO cell.

18. A pharmaceutical preparation comprising the variant ActRIIB polypeptide of any one of claims 1-17 and a pharmaceutically acceptable carrier.

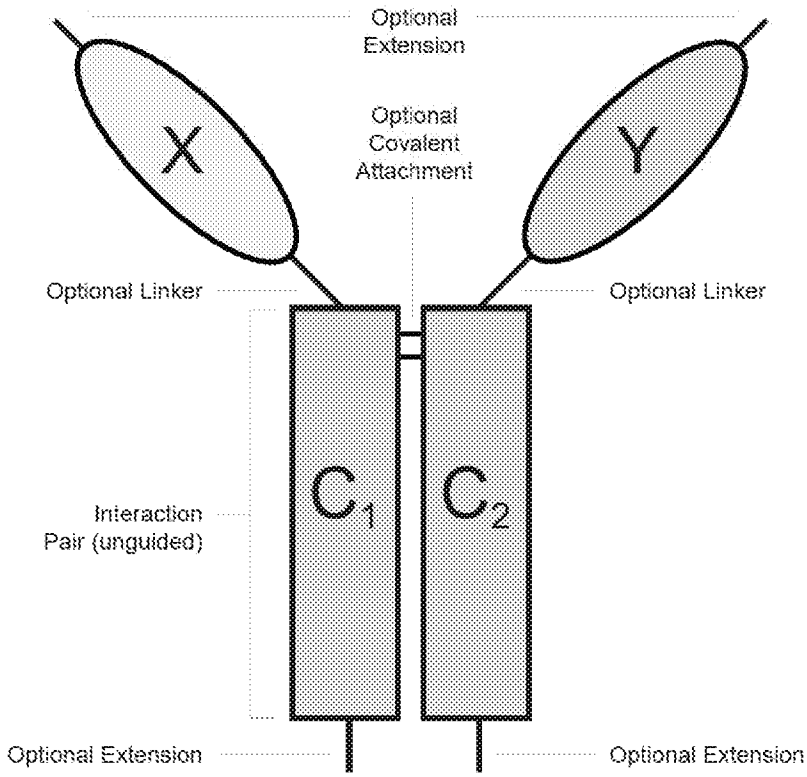
Optional
Extension
X
Optional
Covalent
Attachment
Y
Optional Linker
Optional Linker
C1
C2
Interaction
Pair (unguided)
Optional Extension
Optional Extension

FIGURE 1A

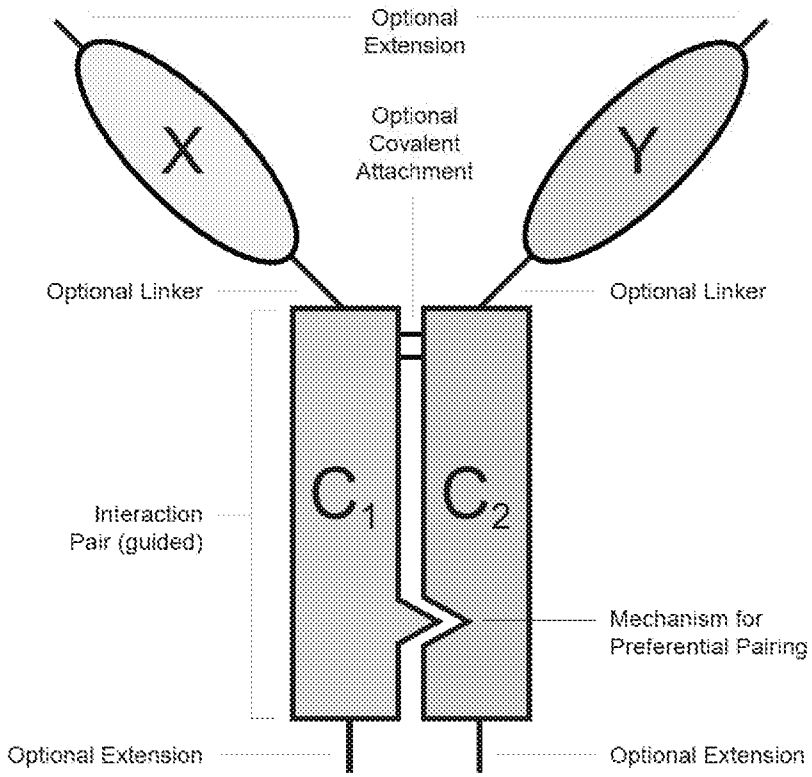
Optional Extension
Optional Covalent Attachment
X
Y
Optional Linker
Optional Linker
C1
C2
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 1B

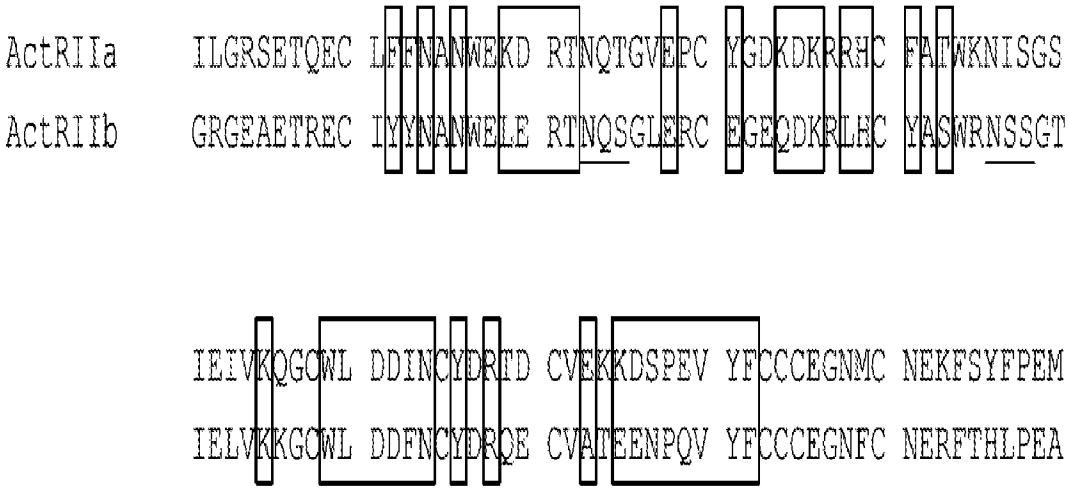
ActRIIa ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS
ActRIIb GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
EVTQPTSNPV TPKPPT
GGPEVTYEPP PTAPT

FIGURE 2

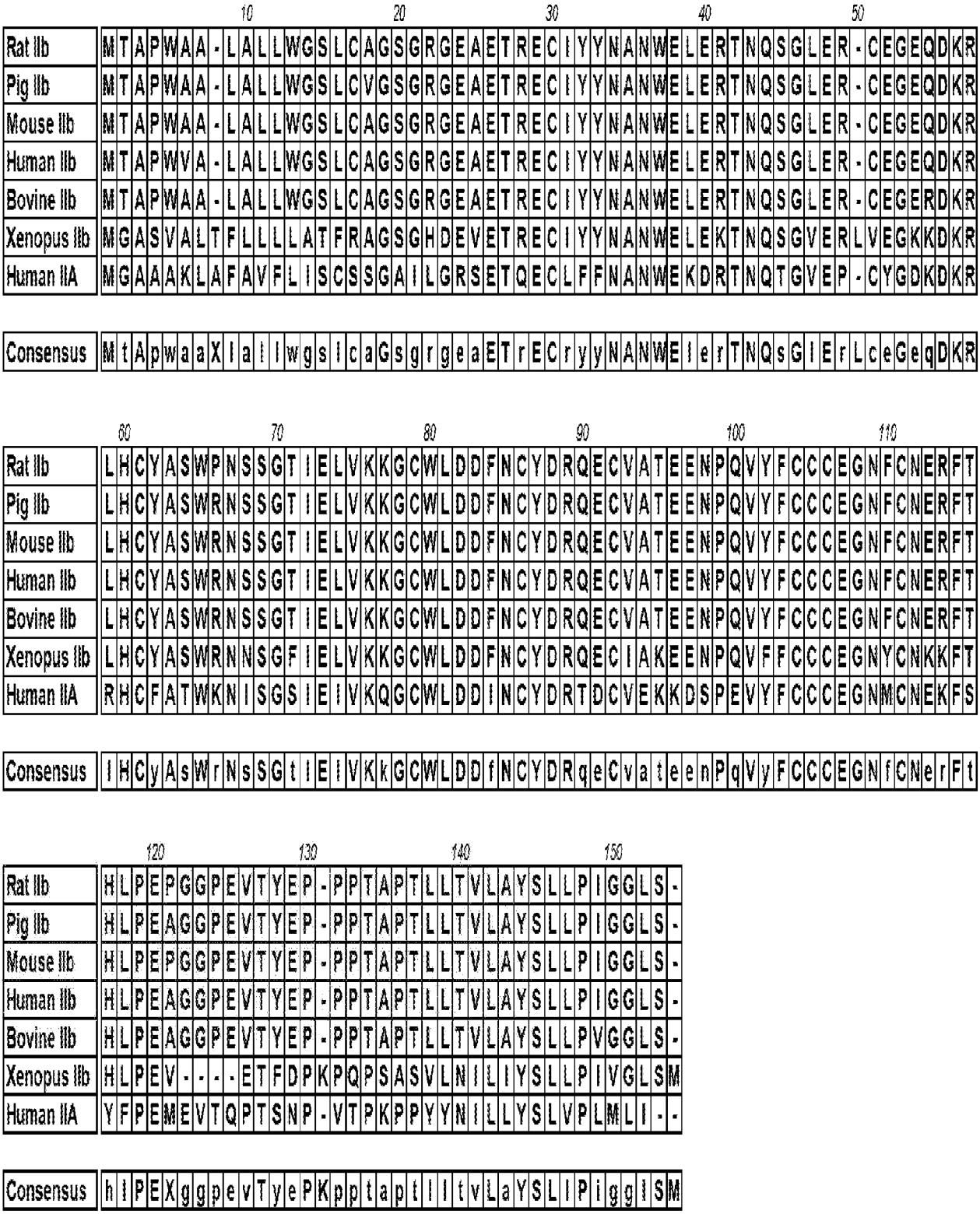
10 20 30 40 50
Rat IIb MTAPWAA-LALLWGSLCAGSGRGEAETRECIYYNANWELERTNQSGLER-CEGEQDKR
Pig IIb MTAPWAA-LALLWGSLCVGSGRGEAETRECIYYNANWELERTNQSGLER-CEGEQDKR
Mouse IIb MTAPWAA-LALLWGSLCAGSGRGEAETRECIYYNANWELERTNQSGLER-CEGEQDKR
Human IIb MTAPWVA-LALLWGSLCAGSGRGEAETRECIYYNANWELERTNQSGLER-CEGEQDKR
Bovine IIb MTAPWAA-LALLWGSLCAGSGRGEAETRECIYYNANWELERTNQSGLER-CEGERDKR
Xenopus IIb MGASVALTFLLLLATFRAGSGHDEVETRECIYYNANWELEKTNQSGVERLVEGKKDKR
Human IIA MGAAAKLAFAVFLISCSSGAILGRSETQECLFFNANWEKDRTNQTGVEP-CYGDKDKR
Consensus MtApwaaXlalllwgslcaGsgrgeaETrECryyNANWElerTNQsGlErLceGeqDKR
60 70 80 90 100 110
Rat IIb LHCYASWPNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFT
Pig IIb LHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFT
Mouse IIb LHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFT
Human IIb LHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFT
Bovine IIb LHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFT
Xenopus IIb LHCYASWRNNSGFIELVKKGCWLDDFNCYDRQECIAKEENPQVFFCCCEGNYCNKKFT
Human IIA RHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFS
Consensus lHCyAsWrNssGtIEIVKkGCWLDDfNCYDRqeCvateenPqVyFCCCEGNfCNerFt
120 130 140 150
Rat IIb HLPEPGGPEVTYEP-PPTAPTLLTVLAYSLLPIGGLS-
Pig IIb HLPEAGGPEVTYEP-PPTAPTLLTVLAYSLLPIGGLS-
Mouse IIb HLPEPGGPEVTYEP-PPTAPTLLTVLAYSLLPIGGLS-
Human IIb HLPEAGGPEVTYEP-PPTAPTLLTVLAYSLLPIGGLS-
Bovine IIb HLPEAGGPEVTYEP-PPTAPTLLTVLAYSLLPVGGLS-
Xenopus IIb HLPEV----ETFDPKPQPSASVLNILIYSLLPIVGLSM
Human IIA YFPEMEVTQPTSNP-VTPKPPYYNILLYSLVPLMLI--
Consensus hlPEXggpevTyePKppptaptlllTvLaYSLIPiggISM

FIGURE 3

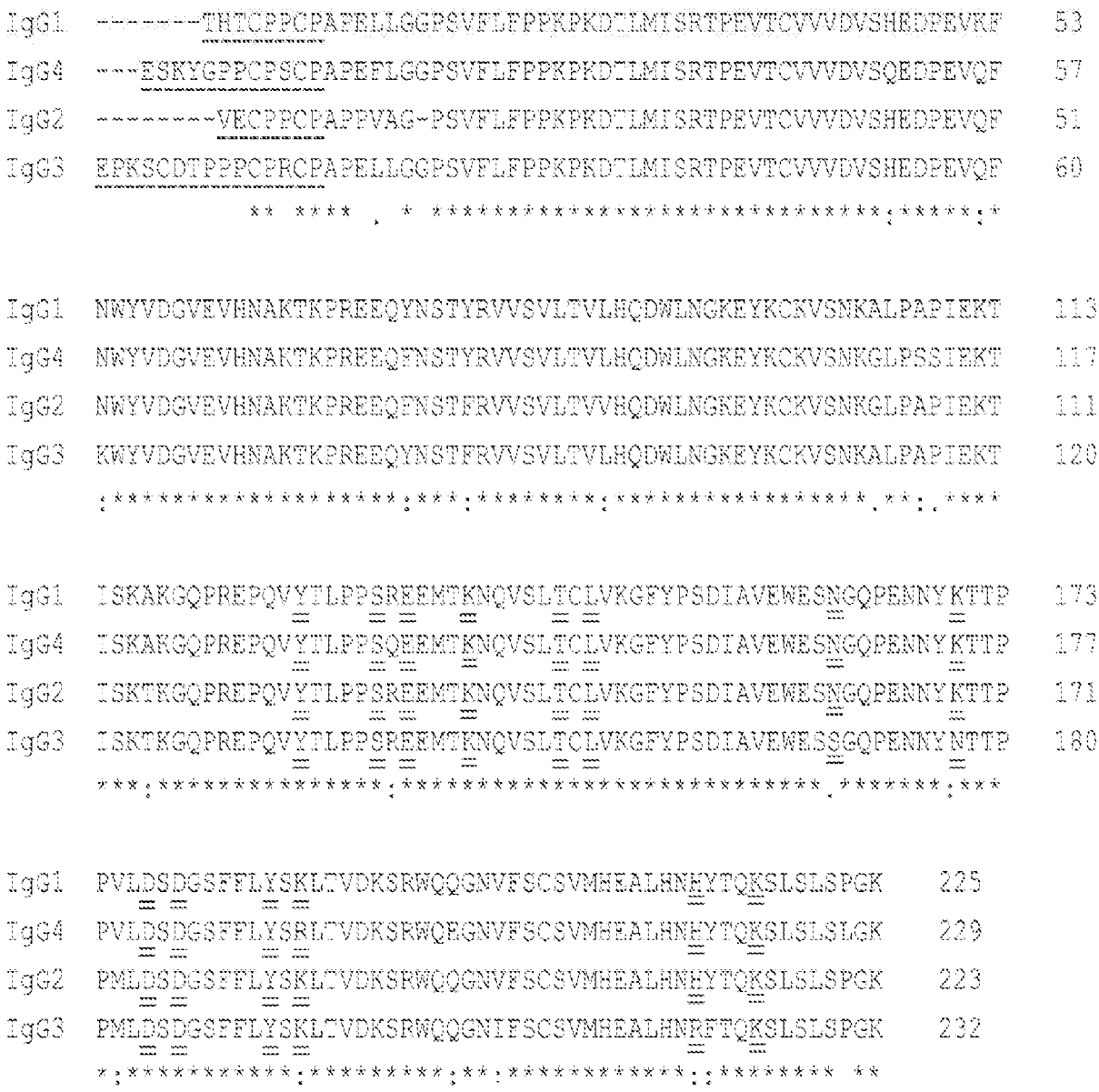
IgG1 -------THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF 53
IgG4 ---ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF 57
IgG2 --------VECPPCPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 51
IgG3 EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 60
IgG1 NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 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 4

```
 20      GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 70      IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
120      GGPEVTYEPP PTAPT    (SEQ ID NO: 1)
```

FIGURE 5

```
  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: 2)
```

FIGURE 6

GGGCGTGGGGAGGCTGAGACACGGGAGTGCATCTACTACAACGCCAACTGGGAGCTGGAGCGCACCAACC
AGAGCGGCCTGGAGCGCTGCGAAGGCGAGCAGGACAAGCGGCTGCACTGCTACGCCTCCTGGCGCAACAG
CTCTGGCACCATCGAGCTCGTGAAGAAGGGCTGCTGGCTAGATGACTTCAACTGCTACGATAGGCAGGAG
TGTGTGGCCACTGAGGAGAACCCCCAGGTGTACTTCTGCTGCTGTGAAGGCAACTTCTGCAACGAGCGCT
TCACTCATTTGCCAGAGGCTGGGGGCCCGGAAGTCACGTACGAGCCACCCCCGACAGCCCCCACC
(SEQ ID NO: 3)

FIGURE 7

ATGACGGCGCCCTGGGTGGCCCTCGCCCTCCTCTGGGGATCGCTGTGGCCCGGCTCTGGGCGTGGGGAGG
CTGAGACACGGGAGTGCATCTACTACAACGCCAACTGGGAGCTGGAGCGCACCAACCAGAGCGGCCTGGA
GCGCTGCGAAGGCGAGCAGGACAAGCGGCTGCACTGCTACGCCTCCTGGCGCAACAGCTCTGGCACCATC
GAGCTCGTGAAGAAGGGCTGCTGGCTAGATGACTTCAACTGCTACGATAGGCAGGAGTGTGTGGCCACTG
AGGAGAACCCCCAGGTGTACTTCTGCTGCTGTGAAGGCAACTTCTGCAACGAGCGCTTCACTCATTTGCC
AGAGGCTGGGGGCCCGGAAGTCACGTACGAGCCACCCCCGACAGCCCCCACCCTGCTCACGGTGCTGGCC
TACTCACTGCTGCCCATCGGGGGCCTTTCCCTCATCGTCCTGCTGGCCTTTTGGATGTACCGGCATCGCA
AGCCCCCCTACGGTCATGTGGACATCCATGAGGACCCTGGGCCTCCACCACCATCCCCTCTGGTGGGCCT
GAAGCCACTGCAGCTGCTGGAGATCAAGGCTCGGGGGCGCTTTGGCTGTGTCTGGAAGGCCCAGCTCATG
AATGACTTTGTAGCTGTCAAGATCTTCCCACTCCAGGACAAGCAGTCGTGGCAGAGTGAACGGGAGATCT
TCAGCACACCTGGCATGAAGCACGAGAACCTGCTACAGTTCATTGCTGCCGAGAAGCGAGGCTCCAACCT
CGAAGTAGAGCTGTGGCTCATCACGGCCTTCCATGACAAGGGCTCCCTCACGGATTACCTCAAGGGGAAC
ATCATCACATGGAACGAACTGTGTCATGTAGCAGAGACGATGTCACGAGGCCTCTCATACCTGCATGAGG
ATGTGCCCTGGTGCCGTGGCGAGGGCCACAAGCCGTCTATTGCCCACAGGGACTTTAAAAGTAAGAATGT
ATTGCTGAAGAGCGACCTCACAGCCGTGCTGGCTGACTTTGGCTTGGCTGTTCGATTTGAGCCAGGGAAA
CCTCCAGGGGACACCCACGGACAGGTAGGCACGAGACGGTACATGGCTCCTGAGGTGCTCGAGGGAGCCA
TCAACTTCCAGAGAGATGCCTTCCTGCGCATTGACATGTATGCCATGGGGTTGGTGCTGTGGGAGCTTGT
GTCTCGCTGCAAGGCTGCAGACGGACCCGTGGATGAGTACATGCTGCCCTTTGAGGAAGAGATTGGCCAG
CACCCTTCGTTGGAGGAGCTGCAGGAGGTGGTGGTGCACAAGAAGATGAGGCCCACCATTAAAGATCACT
GGTTGAAACACCCGGGCCTGGCCCAGCTTTGTGTGACCATCGAGGAGTGCTGGGACCATGATGCAGAGGC
TCGCTTGTCCGCGGGCTGTGTGGAGGAGCGGGTGTCCCTGATTCGGAGGTCGGTCAACGGCACTACCTCG
GACTGTCTCGTTTCCCTGGTGACCTCTGTCACCAATGTGGACCTGCCCCCTAAAGAGTCAAGCATCTAA
(SEQ ID NO: 4)

FIGURE 8

Ligand Binding by Homodimeric ActRIIB-Fc Proteins at 37°C												
ActRIIB protein	Activin A			GDF11			BMP9			BMP10		
	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)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Wild-type	2.3×10^6	1.1×10^{-4}	47	1.0×10^7	1.2×10^{-4}	12	3.0×10^7	1.1×10^{-3}	37	3.6×10^7	1.6×10^{-4}	4
K55A	3.0×10^6	1.4×10^{-4}	46	1.6×10^7	4.0×10^{-4}	26	4.9×10^7	7.0×10^{-3}	142	3.2×10^7	7.4×10^{-4}	23
K55A/F82I	5.7×10^6	2.7×10^{-4}	47	2.2×10^7	2.0×10^{-3}	90	1.6×10^7	2.1×10^{-3}	134	1.3×10^8	8.6×10^{-4}	7
K55E	2.5×10^6	1.6×10^{-4}	64	1.2×10^7	6.3×10^{-4}	52	1.1×10^8	3.0×10^{-2}	270	3.4×10^7	4.7×10^{-4}	14
K74A	3.2×10^7	1.1×10^{-3}	34	1.9×10^6	8.1×10^{-4}	430	1.7×10^8	6.2×10^{-2}	360	7.5×10^7	2.5×10^{-3}	33
L79H	1.9×10^6	5.6×10^{-4}	300	2.0×10^7	7.5×10^{-4}	37	2.4×10^6	1.8×10^{-3}	760	1.9×10^7	2.1×10^{-3}	120
L79H/F82I	1.2×10^6	6.7×10^{-4}	580	2.6×10^7	1.7×10^{-3}	64	1.3×10^7	2.8×10^{-3}	220	2.7×10^7	3.0×10^{-3}	110
L79K	4.0×10^6	5.1×10^{-4}	130	1.2×10^7	1.7×10^{-3}	140	1.6×10^7	1.7×10^{-2}	1100	4.7×10^6	6.5×10^{-3}	1400
L79K/F82K	1.4×10^6	8.6×10^{-4}	640	4.5×10^7	6.7×10^{-2}	1600	No binding			2.2×10^7	1.5×10^{-4}	68
F82I	1.9×10^6	1.5×10^{-4}	78	8.2×10^6	8.4×10^{-5}	10	9.2×10^7	2.5×10^{-2}	275	2.4×10^7	1.5×10^{-4}	8
F82K	1.8×10^6	1.7×10^{-4}	93	1.6×10^7	9.1×10^{-4}	57	Transient binding*			3.1×10^7	2.6×10^{-4}	8
* Indeterminate due to transient nature of interaction												

FIGURE 9

Ligand Binding by Homodimeric ActRIIB-Fc Proteins at 25°C												
ActRIIB protein	Activin A			GDF11			BMP9			BMP10		
	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)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Wild-type	2.3×10^6	5.2×10^{-4}	250	9.1×10^6	9.8×10^{-5}	11	7.4×10^6	4.4×10^{-4}	59	3.3×10^6	5.0×10^{-4}	169
N35E	1.3×10^6	1.0×10^{-3}	800	6.7×10^6	1.9×10^{-4}	28	No binding			4.3×10^6	1.2×10^{-3}	280
N35F	1.6×10^6	4.7×10^{-4}	290	8.2×10^6	1.4×10^{-4}	17	Reduced binding			2.6×10^6	5.8×10^{-4}	220
N35Q	2.0×10^6	6.5×10^{-4}	320	7.7×10^6	1.6×10^{-4}	20	Little binding			2.7×10^6	7.1×10^{-4}	270
L38D	1.7×10^6	4.0×10^{-4}	230	5.8×10^6	1.8×10^{-4}	30	6.6×10^6	4.2×10^{-4}	63	3.3×10^6	4.9×10^{-4}	150
L38Q	1.8×10^6	3.5×10^{-4}	200	7.0×10^6	1.5×10^{-4}	21	7.4×10^6	2.5×10^{-4}	33	3.9×10^6	3.5×10^{-4}	89
L38R	1.9×10^6	4.5×10^{-4}	230	6.4×10^6	4.6×10^{-5}	7	1.4×10^7	5.5×10^{-4}	50	1.6×10^6	1.7×10^{-4}	110
K74M	No binding			No binding			No binding			No binding		
K74T	No binding			No binding			No binding			No binding		
L79W	1.3×10^6	3.2×10^{-4}	260	1.2×10^7	5.2×10^{-4}	44	9.2×10^6	1.1×10^{-3}	110	2.9×10^6	4.7×10^{-4}	160
F82Y	2.3×10^6	3.9×10^{-4}	170	7.1×10^6	1.3×10^{-4}	18	8.4×10^6	6.9×10^{-4}	82	3.7×10^6	5.2×10^{-4}	140
Q98A	3.4×10^6	5.3×10^{-4}	155	4.7×10^6	1.8×10^{-4}	37	1.2×10^7	5.3×10^{-4}	43	2.6×10^6	5.4×10^{-4}	210
Q98I	4.1×10^6	6.4×10^{-4}	157	3.9×10^6	1.9×10^{-4}	49	1.6×10^7	9.4×10^{-4}	59	2.6×10^6	5.6×10^{-4}	210
Q98K	3.3×10^6	4.8×10^{-4}	145	4.6×10^6	1.7×10^{-4}	37	1.1×10^7	7.4×10^{-4}	69	7.4×10^6	5.0×10^{-4}	68
Q98L	3.8×10^6	8.2×10^{-4}	220	4.0×10^6	1.7×10^{-4}	43	1.6×10^7	1.2×10^{-3}	71	1.1×10^7	7.2×10^{-4}	65
Q98R	3.4×10^6	1.0×10^{-3}	300	5.5×10^6	1.1×10^{-4}	20	9.7×10^7	7.2×10^{-3}	74	2.5×10^6	8.1×10^{-4}	320
Q98V	3.6×10^6	6.0×10^{-4}	160	5.0×10^6	1.6×10^{-4}	33	1.1×10^7	5.0×10^{-4}	47	1.0×10^7	5.0×10^{-4}	48

FIGURE 10

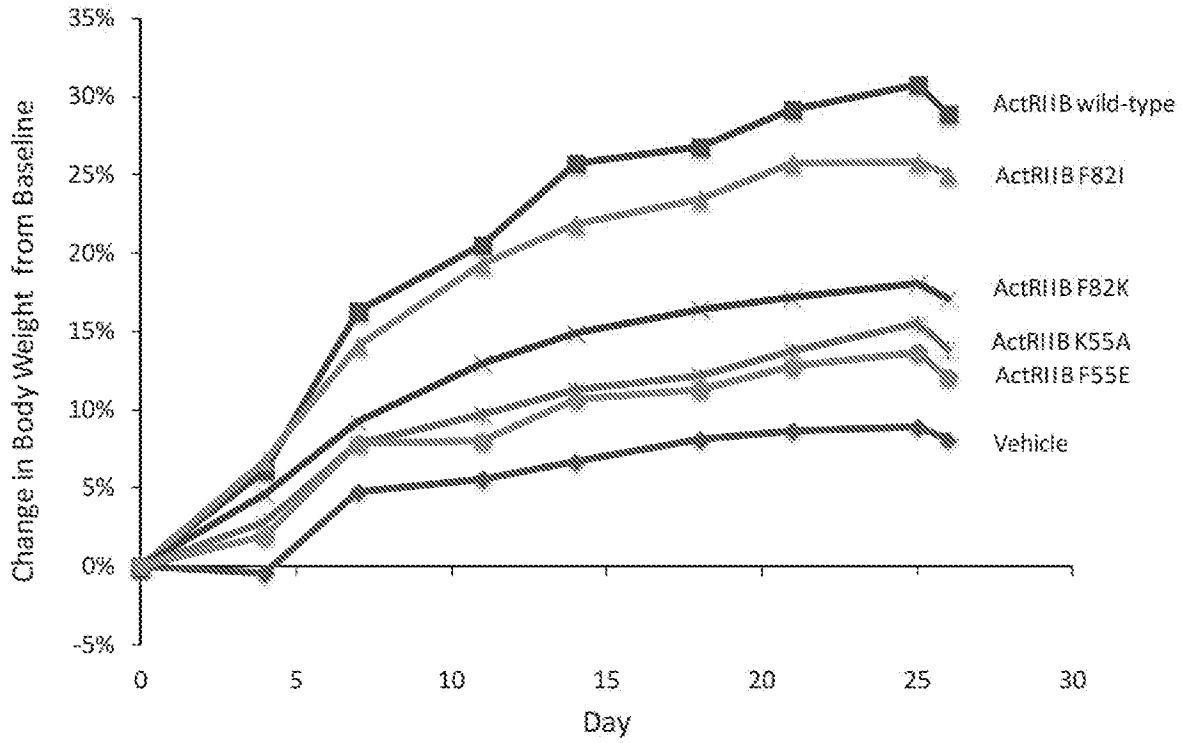
35%
30%
25%
20%
15%
10%
5%
0%
-5%
Change in Body Weight from Baseline
0
5
10
15
20
25
30
Day
ActRIIB wild-type
ActRIIB F82I
ActRIIB F82K
ActRIIB K55A
ActRIIB F55E
Vehicle

FIGURE 11

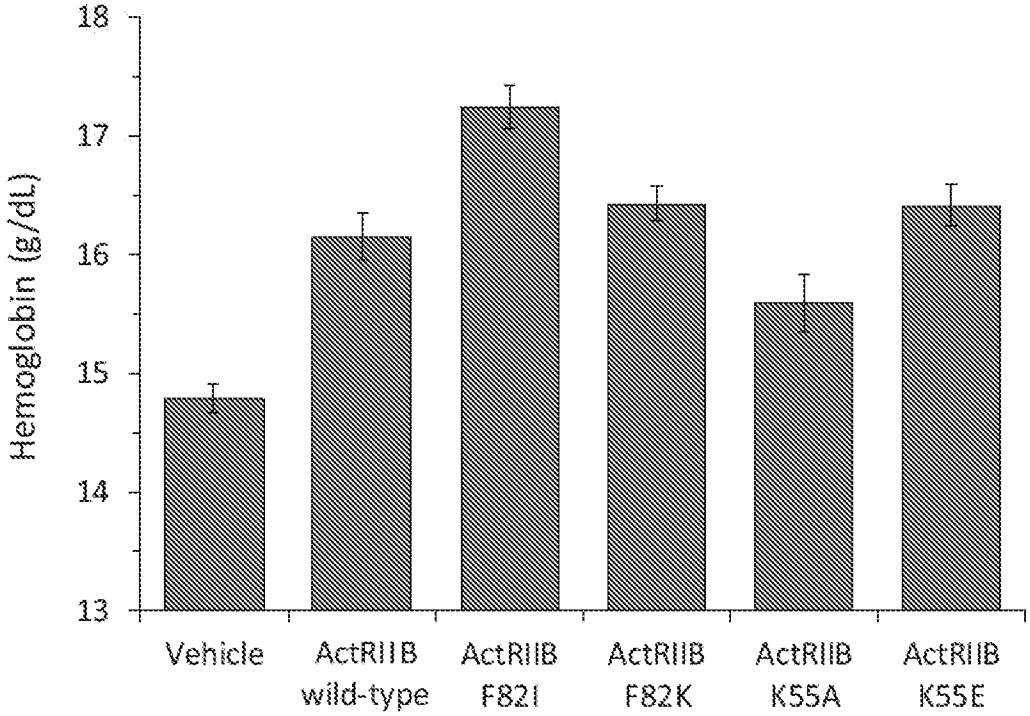
Hemoglobin (g/dL)
18
17
16
15
14
13
Vehicle
ActRIIB wild-type
ActRIIB F82I
ActRIIB F82K
ActRIIB K55A
ActRIIB K55E

FIGURE 12

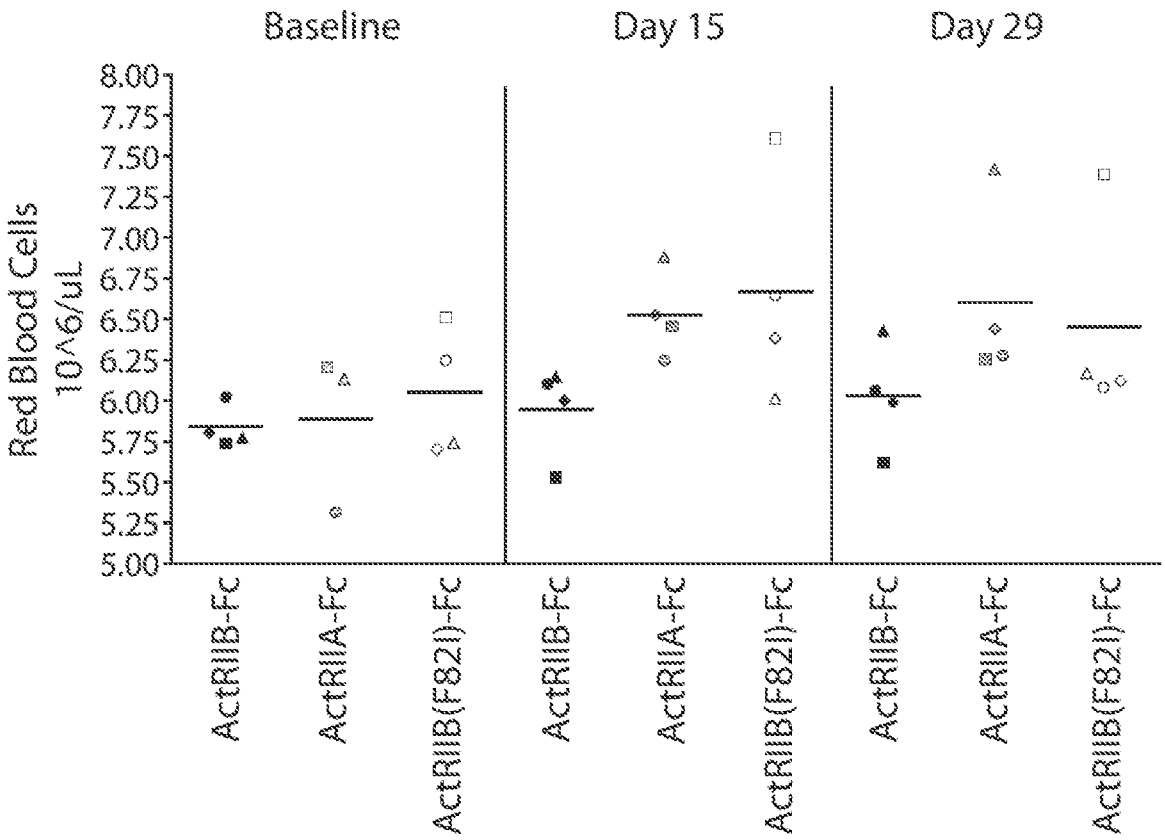
Baseline
Day 15
Day 29
Red Blood Cells
10^6/uL
8.00
7.75
7.50
7.25
7.00
6.75
6.50
6.25
6.00
5.75
5.50
5.25
5.00
ActRIIB-Fc
ActRIIA-Fc
ActRIIB(F82I)-Fc
ActRIIB-Fc
ActRIIA-Fc
ActRIIB(F82I)-Fc
ActRIIB-Fc
ActRIIA-Fc
ActRIIB(F82I)-Fc

FIGURE 13