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(71) Applicant: **KEROS THERAPEUTICS, INC.** [US/US]; One Kendall Square, Building 1400W, Suite 14303, Cambridge, MA 02139 (US).

(72) Inventors: **SEEHRA, Jasbir, S.**; 3 Lincoln Terrace, Lexington, MA 02421 (US). **LACHEY, Jennifer**; 99 Tower Road, Lincoln, MA 01773 (US).

(74) Agent: **BIEKER-BRADY, Kristina**; Clark & Elbing LLP, 101 Federal Street, 15th Floor, Boston, MA 02110 (US).

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(54) Title: ACTIVIN RECEPTOR TYPE IIA VARIANTS AND METHODS OF USE THEREOF

(57) Abstract: The invention features polypeptides that include an extracellular ActRIIa variant. In some embodiments, a polypeptide of the invention includes an extracellular ActRIIa variant fused to an Fc domain monomer or moiety. The invention also features pharmaceutical compositions and methods of using the polypeptides to treat diseases and conditions involving bone damage, e.g., primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet- related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss.

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ACTIVIN RECEPTOR TYPE IIA VARIANTS AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

Healthy bone undergoes a constant remodeling that involves both bone breakdown and bone growth. Bone growth is mediated by the osteoblast cell type whereas the osteoclasts resorb the bone Pathology occurs when these systems fall out of balance either through downregulation of the anabolic program, upregulation of the catabolic system or a combination of both, resulting in a net bone loss. Therefore, controlling the balance in bone remodeling can be useful for promoting the healing of fractures and other damage to bone as well as the treatment of disorders, such as osteoporosis, associated with loss of bone mass and bone mineralization.

Bone damage can result from a range of root causes, including age- or cancer-related bone loss, genetic conditions, adverse side effects of drug treatment, or fracture. The World Health Organization estimates that osteoporosis alone affects 75 million people in the U.S., Europe and Japan, and is a significant risk factor in bone fracture. In general, the whole of bone loss represents pathological states for which there are few effective treatments. Treatment instead focuses on immobilization, exercise and dietary modifications rather than agents that directly promote bone growth and increase bone density. With respect to osteoporosis, estrogen, calcitonin, osteocalcin with vitamin K, or high doses of dietary calcium are all used as therapeutic interventions. Other therapeutic approaches to osteoporosis include bisphosphonates, parathyroid hormone, parathyroid hormone related protein (PTHrP) calcimimetics, statins, anabolic steroids, lanthanum and strontium salts, and sodium fluoride. Such therapeutics, however, are often associated with undesirable side effects. There exists a need for novel and effective treatments for diseases that result in bone damage or bone demineralization.

SUMMARY OF THE INVENTION

The present invention features polypeptides that include an extracellular activin receptor type IIa (ActRIIa) variant. In some embodiments, a polypeptide of the invention includes an extracellular ActRIIa variant fused to the N- or C-terminus of an Fc domain monomer or moiety. Such moieties may be attached by amino acid or other covalent bonds. A polypeptide including an extracellular ActRIIa variant fused to an Fc domain monomer may also form a dimer (e.g., a homodimer or heterodimer) through the interaction between two Fc domain monomers. The polypeptides of the invention may be used to increase bone mass or bone mineral density in a subject having a disease or condition involving bone damage, e.g., primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility.. Further, the polypeptides of the invention may also be used to affect myostatin, activin, and/or bone morphogenetic protein 9 (BMP9) signaling in a subject having a risk of developing or having a disease or condition involving bone damage or bone demineralization.

In one aspect, the invention features a polypeptide including an extracellular activin receptor type IIa (ActRIIa) variant, the variant having a sequence of

GAILGRSETQECLX$_1$X$_2$NANWX$_3$X$_4$X$_5$X$_6$TNQTGVEX$_7$CX$_8$GX$_9$X$_{10}$X$_{11}$X$_{12}$X$_{13}$X$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVX$_{17}$X$_{18}$GCX$_{19}$X$_{20}$X$_{21}$DX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ

ID NO: 1), wherein X_1 is F or Y; X_2 is F or Y; X_3 is E or A; X_4 is K or L; X_5 is D or E; X_6 is R or A; X_7 is P or R; X_8 is Y or E; X_9 is D or E; X_{10} is K or Q; X_{11} is D or A; X_{12} is K or A; X_{13} is R or A; X_{14} is R or L; X_{15} is F or Y; X_{16} is K, R, or A; X_{17} is K, A, Y, F, or I; X_{18} is Q or K; X_{19} is W or A; X_{20} is L or A; X_{21} is D, K, R, A, F, G, M, N, or I; X_{22} is I, F, or A; X_{23} is K or T; X_{24} is K or E; X_{25} is D or E; X_{26} is S or N; and X_{27} is E or Q, and wherein the variant has at least one amino acid substitution relative to a wild-type extracellular ActRIIa having the sequence of SEQ ID NO: 73 or an extracellular ActRIIa having any one of the sequences of SEQ ID NOs: 76-96.

In some embodiments, the variant has a sequence of GAILGRSETQECLFX$_2$NANWX$_3$X$_4$X$_5$X$_6$TNQTGVEX$_7$CX$_8$GX$_9$KX$_{11}$X$_{12}$X$_{13}$X$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVX$_{17}$X$_{18}$GCX$_{19}$X$_{20}$X$_{21}$DX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 2), wherein X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{18}, X_{19}, X_{20}, X_{21}, X_{22}, X_{23}, X_{24}, X_{25}, X_{26}, and X_{27} are defined as above.

In some embodiments, the variant has a sequence of GAILGRSETQECLFX$_2$NANWEX$_4$X$_5$RTNQTGVEX$_7$CX$_8$GX$_9$KDKRX$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVKX$_{18}$GCWLDDX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 3), wherein X_2, X_4, X_5, X_7, X_8, X_9, X_{14}, X_{15}, X_{16}, X_{18}, X_{22}, X_{23}, X_{24}, X_{25}, X_{26}, and X_{27} are defined as above.

In some embodiments, the variant has a sequence of GAILGRSETQECLFX$_2$NANWEX$_4$DRTNQTGVEX$_7$CX$_8$GX$_9$KDKRX$_{14}$HCX$_{15}$ATWX^{16}NISGSIEIVKX$_{18}$GCWLDDX$_{22}$NCYDRTDCVEX$_{23}$KX$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 4), wherein X_2, X_4, X_7, X_8, X_9, X_{14}, X_{15}, X^{16}, X_{18}, X_{22}, X_{23}, X_{25}, X_{26}, and X_{27} are defined as above.

In some embodiments, the variant has a sequence of GAILGRSETQECLFX$_2$NANWEX$_4$DRTNQTGVEPCX$_8$GX$_9$KDKRX$_{14}$HCFATWKNISGSIEIVKX$_{18}$GCWLDDINCYDRTDCVEX$_{23}$KX$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 5), wherein X_2, X_4, X_8, X_9, X_{14}, X_{18}, X_{23}, X_{25}, X_{26}, and X_{27} are defined as above.

In any of the aforementioned embodiments, X_1 is F or Y. In any of the aforementioned embodiments, X_2 is F or Y. In any of the aforementioned embodiments, X_3 is E or A. In any of the aforementioned embodiments, X_4 is K or L. In any of the aforementioned embodiments, X_5 is D or E. In any of the aforementioned embodiments, X_6 is R or A. In any of the aforementioned embodiments, X_7 is P or R. In any of the aforementioned embodiments, X_8 is Y or E. In any of the aforementioned embodiments, X_9 is D or E. In any of the aforementioned embodiments, X_{10} is K or Q. In any of the aforementioned embodiments, X_{11} is D or A. In any of the aforementioned embodiments, X_{12} is K or A. In any of the aforementioned embodiments, X_{13} is R or A. In any of the aforementioned embodiments, X_{14} is R or L. In any of the aforementioned embodiments, X_{15} is F or Y. In any of the aforementioned embodiments, X_{16} is K, R, or A. In any of the aforementioned embodiments, X_{17} is K, A, Y, F, or I. In any of the aforementioned embodiments, X_{18} is Q or K. In any of the aforementioned embodiments, X_{19} is W or A. In any of the aforementioned embodiments, X_{20} is L or A. In any of the aforementioned embodiments, X_{21} is D, K, R, A, F, G, M, N, or I. In any of the aforementioned embodiments, X_{22} is I, F, or A. In any of the aforementioned embodiments, X_{23} is K or T. In any of the aforementioned embodiments, X_{24} is K or E. In any of the aforementioned embodiments, X_{25} is D or E. In any of the aforementioned embodiments, X_{26} is S or N. In any of the aforementioned embodiments, X_{27} is E or Q. In any of the

aforementioned embodiments, X_{23} is T, X_{24} is E, X_{25} is E, and X_{26} is N or X_{23} is T, X_{24} is K, X_{25} is E, and X_{26} is N. In any of the aforementioned embodiments, X_{17} is K.

In any of the aforementioned embodiments, the variant has the sequence of any one of SEQ ID NOs: 6-72.

In any of the aforementioned embodiments, the amino acid at position X_{24} may be replaced with the amino acid K.

In any of the aforementioned embodiments, the amino acid at position X_{24} may be replaced with the amino acid E.

In any of the aforementioned embodiments, a polypeptide described herein may further include a C-terminal extension of one or more amino acids (e.g., 1, 2, 3, 4, 5, 6 more amino acids). In some embodiments, the C-terminal extension is amino acid sequence NP. In some embodiments, the C-terminal extension is amino acid sequence NPVTPK (SEQ ID NO: 155).

In any of the aforementioned embodiments, a polypeptide described herein may further include a moiety fused or covalently linked to the C-terminus of the polypeptide. The moiety may increase the stability of improve pharmacokinetic properties of the polypeptide. In some embodiments, the moiety is an Fc domain monomer, an Fc domain, an albumin binding peptide, a fibronectin domain, or serum albumin.

In any of the aforementioned embodiments, a polypeptide described herein may further include an Fc domain monomer fused to the C-terminus of the polypeptide by way of a linker. In some embodiments, the polypeptide that includes an extracellular ActRIIa variant described herein fused to an Fc domain monomer may form a dimer (e.g., a homodimer or heterodimer) through the interaction between two Fc domain monomers. In some embodiments, the Fc domain monomer has the sequence of SEQ ID NO: 97

In any of the aforementioned embodiments, a polypeptide described herein may further include an Fc domain fused to the C-terminus of the polypeptide by way of a linker. In some embodiments, the Fc domain is a wild-type Fc domain. In some embodiments, the wild-type Fc domain has the sequence of SEQ ID NO: 151. In some embodiments, the Fc domain contains one or more amino acid substitutions. In some embodiments, the Fc domain containing one or more amino acid substitutions does not form a dimer.

In any of the aforementioned embodiments, a polypeptide described herein may further include an albumin-binding peptide fused to the C-terminus of the polypeptide by way of a linker. In some embodiments, the albumin-binding peptide has the sequence of SEQ ID NO: 152.

In any of the aforementioned embodiments, a polypeptide described herein may further include a fibronectin domain fused to the C-terminus of the polypeptide by way of a linker. In some embodiments, the fibronectin domain peptide has the sequence of SEQ ID NO: 153.

In any of the aforementioned embodiments, a polypeptide described herein may further include a human serum albumin fused to the C-terminus of the polypeptide by way of a linker. In some embodiments, the human serum albumin has the sequence of SEQ ID NO: 154.

In some embodiments, the linker is an amino acid spacer. In some embodiments, the amino acid spacer is GGG, GGGA (SEQ ID NO: 98), GGGG (SEQ ID NO: 100), GGGAG (SEQ ID NO: 130), GGGAGG (SEQ ID NO: 131), or GGGAGGG (SEQ ID NO: 132).

In some embodiments, the amino acid spacer is GGGS (SEQ ID NO: 99), GGGGA (SEQ ID NO: 101), GGGGS (SEQ ID NO: 102), GGGGG (SEQ ID NO: 103), GGAG (SEQ ID NO: 104), GGSG (SEQ ID NO: 105), AGGG (SEQ ID NO: 106), SGGG (SEQ ID NO: 107), GAGA (SEQ ID NO: 108), GSGS (SEQ ID NO: 109), GAGAGA (SEQ ID NO: 110), GSGSGS (SEQ ID NO: 111), GAGAGAGA (SEQ ID NO: 112), GSGSGSGS (SEQ ID NO: 113), GAGAGAGAGA (SEQ ID NO: 114), GSGSGSGSGS (SEQ ID NO: 115), GAGAGAGAGAGA (SEQ ID NO: 116), and GSGSGSGSGSGS (SEQ ID NO: 117), GGAGGA (SEQ ID NO: 118), GGSGGS (SEQ ID NO: 119), GGAGGAGGA (SEQ ID NO: 120), GGSGGSGGS (SEQ ID NO: 121), GGAGGAGGAGGA (SEQ ID NO: 122), GGSGGSGGSGGS (SEQ ID NO: 123), GGAGGGAG (SEQ ID NO: 124), GGSGGGSG (SEQ ID NO: 125), GGAGGGAGGGAG (SEQ ID NO: 126), and GGSGGGSGGGSG (SEQ ID NO: 127), GGGGAGGGGAGGGGA (SEQ ID NO: 128), GGGGSGGGGSGGGGS (SEQ ID NO: 129), AAAL (SEQ ID NO: 133), AAAK (SEQ ID NO: 134), AAAR (SEQ ID NO: 135), EGKSSGSGSESKST (SEQ ID NO: 136), GSAGSAAGSGEF (SEQ ID NO: 137), AEAAAKEAAAKA (SEQ ID NO: 138), KESGSVSSEQLAQFRSLD (SEQ ID NO: 139), GENLYFQSGG (SEQ ID NO: 140), SACYCELS (SEQ ID NO: 141), RSIAT (SEQ ID NO: 142), RPACKIPNDLKQKVMNH (SEQ ID NO: 143), GGSAGGSGSGSSGGSSGASGTGTAGGTGSGSGTGSG (SEQ ID NO: 144), AAANSSIDLISVPVDSR (SEQ ID NO: 145), GGSGGGSEGGGSEGGGSEGGGSEGGGSEGGGSGGGS (SEQ ID NO: 146), EAAAK (SEQ ID NO: 147), or PAPAP(SEQ ID NO: 148).

In any of the aforementioned embodiments, the polypeptide described herein has a serum half-life of at least 7 days.

In any of the aforementioned embodiments, the polypeptide described herein binds to human bone morphogenetic protein 9 (BMP9) with a K_D of 200 pM or higher. In some embodiments, the polypeptide binds to activin and/or myostatin and has reduced (e.g., weak) binding to human BMP9. In some embodiments, the polypeptide does not substantially bind to human BMP9.

In any of the aforementioned embodiments, the polypeptide described herein binds to human activin A with a K_D of 800 pM or less.

In any of the aforementioned embodiments, the polypeptide described herein binds to human activin B with a K_D of approximately 800 pM or less.

In any of the aforementioned embodiments, the polypeptide described herein binds to human GDF-11 with a K_D of approximately 5 pM or higher.

In another aspect, the invention features a nucleic acid molecule encoding a polypeptide described herein (e.g., a polypeptide including an extracellular ActRIIa variant having a sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)). In another aspect, the invention also features a vector including the nucleic acid molecule described herein.

In another aspect, the invention features a host cell that expresses a polypeptide described herein, wherein the host cell includes a nucleic acid molecule or a vector described in the previous two aspects, wherein the nucleic acid molecule or vector is expressed in the host cell.

In another aspect, the invention features a method of preparing a polypeptide described herein, wherein the method includes: a) providing a host cell including a nucleic acid molecule or a vector described herein, and b) expressing the nucleic acid molecule or vector in the host cell under conditions that allow for the formation of the polypeptide.

In another aspect, the invention features a pharmaceutical composition including a polypeptide, nucleic acid molecule, or vector described herein and one or more pharmaceutically acceptable carriers or excipients. In some embodiments of the pharmaceutical composition, the polypeptide, nucleic acid molecule, or vector is in a therapeutically effective amount.

In another aspect, the invention also features a construct including two identical polypeptides (e.g., a homodimer) each including an extracellular ActRIIa variant having a sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) fused to the N- or C-terminus of an Fc domain monomer (e.g., the sequence of SEQ ID NO: 97). The two Fc domain monomers in the two polypeptides interact to form an Fc domain in the construct.

In another aspect, the invention also features a construct including two different polypeptides (e.g., a heterodimer) each including an extracellular ActRIIa variant having a sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) fused to the N- or C-terminus of an Fc domain monomer. The two Fc domain monomers in the two polypeptides interact to form an Fc domain in the construct.

In another aspect, the invention features a method of increasing bone mineral density in a subject in need thereof. The method includes administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of reducing bone resorption in a subject in need thereof. The method includes administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of increasing bone formation in a subject in need thereof. The method includes administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In some embodiments of any of the above aspects, the subject has primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss.

In another aspect, the invention features a method of affecting myostatin, activin, and/or BMP9 signaling (e.g., reducing or inhibiting the binding of myostatin, activin, and/or BMP9 to their receptors) in a subject having a disease or condition involving bone damage, wherein method includes administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein. In some embodiments of this aspect, the disease or condition is primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss.

In another aspect, the invention features a method of treating a subject having primary osteoporosis by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having secondary osteoporosis by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having osteopenia by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having a fracture by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having bone cancer or cancer metastasis-related bone loss by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having Paget's disease by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject renal osteodystrophy by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having treatment-related bone loss by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having diet-related bone loss by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having bone loss associated with the treatment of obesity by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having low gravity-related bone loss by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In another aspect, the invention features a method of treating a subject having immobility-related bone loss by administering to the subject a therapeutically effective amount of a polypeptide, nucleic acid molecule, or vector described herein or a pharmaceutical composition described herein.

In some embodiments of any of the above aspects, the primary osteoporosis is age-related osteoporosis.

In some embodiments of any of the above aspects, the primary osteoporosis is hormone-related osteoporosis.

In some embodiments of any of the above aspects, the secondary osteoporosis is immobilization-induced osteoporosis.

In some embodiments of any of the above aspects, wherein the secondary osteoporosis is glucocorticoid-induced osteoporosis.

In some embodiments of any of the above aspects, the cancer is multiple myeloma.

In some embodiments of any of the above aspects, the treatment is FGF-21 treatment.

In some embodiments of any of the above aspects, the treatment is GLP-1 treatment.

In some embodiments of any of the above aspects, the treatment is cancer therapy.

In some embodiments of any of the above aspects, the treatment is treatment for obesity and/or Type-2 diabetes.

In some embodiments of any of the above aspects, the diet-related bone loss is rickets.

In some embodiments of any of the above aspects, the method increases bone formation in the subject. In some embodiments of any of the above aspects, wherein the method decreases bone resorption in the subject. In some embodiments of any of the above aspects, the method increases osteoblast activity or osteoblastogenesis. In some embodiments of any of the above aspects, the method decreases osteoclast activity or decreases osteoclastogenesis. In some embodiments of any of the above aspects, the method reduces or inhibits the binding of activin and/or myostatin to their receptors.

In some embodiments of any of the methods described herein, the method does not cause a vascular complication (e.g., an increase vascular permeability or leakage) in the subject. In some embodiments of any of the methods described herein, the method increases bone mineral density in the subject.

In some embodiments of any of the above aspects, the bone is cortical bone. In some embodiments of any of the above aspects, the bone is trabecular bone.

In some embodiments of any of the above aspects, the polypeptide, nucleic acid, vector, or pharmaceutical composition is administered in an amount sufficient to increase bone density, reduce bone resorption, reduce the rate of bone resorption, increase bone formation, increase the rate of bone formation, reduce osteoclast activity, increase osteoblast activity, or affect myostatin, activin, and/or BMP9 signaling in the subject.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 69. In some embodiments, the variant having the sequence of SEQ ID NO: 69 has the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids at the C-terminus, e.g., the amino acids NP or NPVTPK (SEQ ID NO: 155)). In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a

subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 69, optionally having the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension, or a pharmaceutical composition containing said variant.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 58. In some embodiments, the variant having the sequence of SEQ ID NO: 58 has the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids at the C-terminus, e.g., the amino acids NP or NPVTPK (SEQ ID NO: 155)). In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 58, optionally having the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension, or a pharmaceutical composition containing said variant.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 6. In some embodiments, the variant having the sequence of SEQ ID NO: 6 has the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids at the C-terminus, e.g., the amino acids NP or NPVTPK (SEQ ID NO: 155)). In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 6, optionally having the amino acid K at position

X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension, or a pharmaceutical composition containing said variant.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 38. In some embodiments, the variant having the sequence of SEQ ID NO: 38 has the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids at the C-terminus, e.g., the amino acids NP or NPVTPK (SEQ ID NO: 155 In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 38, optionally having the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension, or a pharmaceutical composition containing said variant.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 41. In some embodiments, the variant having the sequence of SEQ ID NO: 41 has the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids at the C-terminus, e.g., the amino acids NP or NPVTPK (SEQ ID NO: 155)). In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 41, optionally having the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension, or a pharmaceutical composition containing said variant.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 44. In some embodiments, the variant having the sequence of SEQ ID NO: 44 has the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-

terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids at the C-terminus, e.g., the amino acids NP or NPVTPK (SEQ ID NO: 155)). In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 44, optionally having the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension, or a pharmaceutical composition containing said variant.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 70. In some embodiments, the variant having the sequence of SEQ ID NO: 70 has the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids at the C-terminus, e.g., the amino acids NP or NPVTPK (SEQ ID NO: 155)). In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 70, optionally having the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension, or a pharmaceutical composition containing said variant.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 71. In some embodiments, the variant having the sequence of SEQ ID NO: 71 has the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids at the C-terminus, e.g., the amino acids VTPK). In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related

bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 71, optionally having the amino acid K at position X_{17}, the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, and/or a C-terminal extension, or a pharmaceutical composition containing said variant.

In some embodiments of any of the above aspects, the variant has the sequence of SEQ ID NO: 72. In some embodiments, the variant having the sequence of SEQ ID NO: 72 has the amino acid K at position X_{17} and/or the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}. In some embodiments of any of the above aspects, the method includes increasing bone mineral density, increasing bone formation, decreasing bone resorption, or treating a condition or disease involving bone damage in a subject in need thereof (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or affecting myostatin, activin, and/or BMP9 signaling in a subject (e.g., a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) by administering to the subject a therapeutically effective amount of a variant having the sequence of SEQ ID NO: 72, optionally having the amino acid K at position X_{17} and/or the amino acid sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}.

Definitions

As used herein, the term "extracellular activin receptor type IIa (ActRIIa) variant" refers to a peptide including a soluble, extracellular portion of the single transmembrane receptor, ActRIIa, that has at least one amino acid substitution relative to a wild-type extracellular ActRIIa (e.g., bold portion of the sequence of SEQ ID NO: 75 shown below) or an extracellular ActRIIa having any one of the sequences of SEQ ID NOs: 76-96. The sequence of the wild-type, human ActRIIa precursor protein is shown below (SEQ ID NO: 75), in which the signal peptide is italicized and the extracellular portion is bold.

Wild-type, human ActRIIa precursor protein (SEQ ID NO: 75):

*MGAAAKLAFAVFLISCSS***GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFAT**
WKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPT
SNPVTPKPPYYNILLYSLVPLMLIAGIVICAFWVYRHHKMAYPPVLVPTQDPGPPPPSPLLGLKPL
QLLEVKARGRFGCVWKAQLLNEYVAVKIFPIQDKQSWQNEYEVYSLPGMKHENILQFIGAEKRG
TSVDVDLWLITAFHEKGSLSDFLKANVVSWNELCHIAETMARGLAYLHEDIPGLKDGHKPAISHR
DIKSKNVLLKNNLTACIADFGLALKFEAGKSAGDTHGQVGTRRYMAPEVLEGAINFQRDAFLRID
MYAMGLVLWELASRCTAADGPVDEYMLPFEEEIGQHPSLEDMQEVVVHKKKRPVLRDYWQKH
AGMAMLCETIEECWDHDAEARLSAGCVGERITQMQRLTNIITTEDIVTVVTMVTNVDFPPKESSL

An extracellular ActRIIa variant may have a sequence of any one of SEQ ID NOs: 1-72. In particular embodiments, an extracellular ActRIIa variant has a sequence of any one of SEQ ID NOs: 6-72 (Table 2). In some embodiments, an extracellular ActRIIa variant may have at least 85% (e.g., at least 85%, 87%, 90%, 92%, 95%, 97%, or greater) amino acid sequence identity to the sequence of a wild-type extracellular ActRIIa (SEQ ID NO: 73).

As used herein, the term "extracellular ActRIIb variant" refers to a peptide including a soluble, extracellular portion of the single transmembrane receptor, ActRIIb, that has at least one amino acid substitution relative to a wild-type extracellular ActRIIb (e.g., the sequence of SEQ ID NO: 74). An extracellular ActRIIb variant may have the sequence of SEQ ID NO: 149 shown below:

extracellular ActRIIb variant (SEQ ID NO: 149):

GRGEAETRECIFYNANWEKDRTNQSGLEPCYGDQDKRRHCFASWKNSSGTIELVKQGCWLDDI
NCYDRQECVAKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT

As used herein, the term "linker" refers to a linkage between two elements, e.g., peptides or protein domains. A polypeptide described herein may include an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having a sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) fused to a moiety. The moiety may increase stability or improve pharmacokinetic properties of the polypeptide. The moiety (e.g., Fc domain monomer, a wild-type Fc domain, an Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a human serum albumin) may be fused to the polypeptide by way of a linker. A linker can be a covalent bond or a spacer. The term "bond" refers to a chemical bond, e.g., an amide bond or a disulfide bond, or any kind of bond created from a chemical reaction, e.g., chemical conjugation. The term "spacer" refers to a moiety (e.g., a polyethylene glycol (PEG) polymer) or an amino acid sequence (e.g., a 1-200 amino acid sequence) occurring between two elements, e.g., peptides or protein domains, to provide space and/or flexibility between the two elements. An amino acid spacer is part of the primary sequence of a polypeptide (e.g., fused to the spaced peptides via the polypeptide backbone). The formation of disulfide bonds, e.g., between two hinge regions that form an Fc domain, is not considered a linker.

As used herein, the term "Fc domain" refers to a dimer of two Fc domain monomers. An Fc domain has at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, or 100% sequence

identity) to a human Fc domain that includes at least a C_H2 domain and a C_H3 domain. An Fc domain monomer includes second and third antibody constant domains (C_H2 and C_H3). In some embodiments, the Fc domain monomer also includes a hinge domain. An Fc domain does not include any portion of an immunoglobulin that is capable of acting as an antigen-recognition region, e.g., a variable domain or a complementarity determining region (CDR). In the wild-type Fc domain, the two Fc domain monomers dimerize by the interaction between the two C_H3 antibody constant domains, as well as one or more disulfide bonds that form between the hinge domains of the two dimerizing Fc domain monomers. In some embodiments, an Fc domain may be mutated to lack effector functions, typical of a "dead Fc domain." In certain embodiments, each of the Fc domain monomers in an Fc domain includes amino acid substitutions in the C_H2 antibody constant domain to reduce the interaction or binding between the Fc domain and an Fcγ receptor. In some embodiments, the Fc domain contains one or more amino acid substitutions that reduce or inhibit Fc domain dimerization. An Fc domain can be any immunoglobulin antibody isotype, including IgG, IgE, IgM, IgA, or IgD. Additionally, an Fc domain can be an IgG subtype (e.g., IgG1, IgG2a, IgG2b, IgG3, or IgG4). The Fc domain can also be a non-naturally occurring Fc domain, e.g., a recombinant Fc domain.

As used herein, the term "albumin-binding peptide" refers to an amino acid sequence of 12 to 16 amino acids that has affinity for and functions to bind serum albumin. An albumin-binding peptide can be of different origins, e.g., human, mouse, or rat. In some embodiments, an albumin-binding peptide has the sequence DICLPRWGCLW (SEQ ID NO: 152).

As used herein, the term "fibronectin domain" refers to a high molecular weight glycoprotein of the extracellular matrix, or a fragment thereof, that binds to, e.g., membrane-spanning receptor proteins such as integrins and extracellular matrix components such as collagens and fibrins. In some embodiments, a fibronectin domain is a fibronectin type III domain (SEQ ID NO: 153) having amino acids 610-702 of the sequence of UniProt ID NO: P02751. In other embodiments, a fibronectin domain is an adnectin protein.

As used herein, the term "human serum albumin" refers to the albumin protein present in human blood plasma. Human serum albumin is the most abundant protein in the blood. It constitutes about half of the blood serum protein. In some embodiments, a human serum albumin has the sequence of UniProt ID NO: P02768 (SEQ ID NO: 154).

As used herein, the term "fused" is used to describe the combination or attachment of two or more elements, components, or protein domains, e.g., peptides or polypeptides, by means including chemical conjugation, recombinant means, and chemical bonds, e.g., amide bonds. For example, two single peptides in tandem series can be fused to form one contiguous protein structure, e.g., a polypeptide, through chemical conjugation, a chemical bond, a peptide linker, or any other means of covalent linkage. In some embodiments of a polypeptide described herein, an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be fused in tandem series to the N- or C-terminus of a moiety (e.g., Fc domain monomer (e.g., the sequence of SEQ ID NO: 97) a wild-type Fc domain (e.g., the sequence of SEQ ID NO: 151), an Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide (e.g., the sequence of SEQ ID NO: 152), a fibronectin domain (e.g., the sequence of SEQ ID NO: 153), or a human serum albumin (e.g., the sequence of SEQ ID NO: 154)) by way of a linker. For example, an extracellular ActRIIa variant is fused to a moiety (e.g., an Fc

domain monomer, a wild-type Fc domain, an Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a human serum albumin) by way of a peptide linker, in which the N-terminus of the peptide linker is fused to the C-terminus of the extracellular ActRIIa variant through a chemical bond, e.g., a peptide bond, and the C-terminus of the peptide linker is fused to the N-terminus of the moiety (e.g., Fc domain monomer, wild-type Fc domain, Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), albumin-binding peptide, fibronectin domain, or human serum albumin) through a chemical bond, e.g., a peptide bond.

As used herein, the term "C-terminal extension" refers to the addition of one or more amino acids to the C-terminus of a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-70 (e.g., SEQ ID NOs: 6-70)). The C-terminal extension can be one or more amino acids (e.g., 1, 2, 3, 4, 5, 6, or more amino acids). Exemplary C-terminal extensions are the amino acid sequence NP (a two amino acid C-terminal extension) and the amino acid sequence NPVTPK (SEQ ID NO: 155) (a six amino acid C-terminal extension). Any amino acid sequence that does not disrupt the activity of the polypeptide can be used. SEQ ID NO: 71, which is the sequence of SEQ ID NO: 69 with a C-terminal extension of NP, and SEQ ID NO: 72, which is the sequence of SEQ ID NO: 69 with a C-terminal extension of NPVTPK, represent two of the possible ways that a polypeptide of the invention can be modified to include a C-terminal extension.

As used herein, the term "percent (%) identity" refers to the percentage of amino acid (or nucleic acid) residues of a candidate sequence, e.g., an extracellular ActRIIa variant, that are identical to the amino acid (or nucleic acid) residues of a reference sequence, e.g., a wild-type extracellular ActRIIa (e.g., SEQ ID NO: 73), after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity (i.e., gaps can be introduced in one or both of the candidate and reference sequences for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). Alignment for purposes of determining percent identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN, or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. In some embodiments, the percent amino acid (or nucleic acid) sequence identity of a given candidate sequence to, with, or against a given reference sequence (which can alternatively be phrased as a given candidate sequence that has or includes a certain percent amino acid (or nucleic acid) sequence identity to, with, or against a given reference sequence) is calculated as follows:

$$100 \times (\text{fraction of } A/B)$$

where A is the number of amino acid (or nucleic acid) residues scored as identical in the alignment of the candidate sequence and the reference sequence, and where B is the total number of amino acid (or nucleic acid) residues in the reference sequence. In some embodiments where the length of the candidate sequence does not equal to the length of the reference sequence, the percent amino acid (or nucleic acid) sequence identity of the candidate sequence to the reference sequence would not equal to the percent amino acid (or nucleic acid) sequence identity of the reference sequence to the candidate sequence.

In particular embodiments, a reference sequence aligned for comparison with a candidate sequence may show that the candidate sequence exhibits from 50% to 100% identity across the full length of the candidate sequence or a selected portion of contiguous amino acid (or nucleic acid) residues of the candidate sequence. The length of the candidate sequence aligned for comparison purpose is at least 30%, e.g., at least 40%, e.g., at least 50%, 60%, 70%, 80%, 90%, or 100% of the length of the reference sequence. When a position in the candidate sequence is occupied by the same amino acid (or nucleic acid) residue as the corresponding position in the reference sequence, then the molecules are identical at that position.

As used herein, the term "serum half-life" refers to, in the context of administering a therapeutic protein to a subject, the time required for plasma concentration of the protein in the subject to be reduced by half. The protein can be redistributed or cleared from the bloodstream, or degraded, e.g., by proteolysis. As described herein, a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having a sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) displays a serum half-life of 7 days in humans. As used herein, the term "affinity" or "binding affinity" refers to the strength of the binding interaction between two molecules. Generally, binding affinity refers to the strength of the sum total of non-covalent interactions between a molecule and its binding partner, such as an extracellular ActRIIa variant and BMP9 or activin A. Unless indicated otherwise, binding affinity refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of a binding pair. The binding affinity between two molecules is commonly described by the dissociation constant (K_D) or the affinity constant (K_A). Two molecules that have low binding affinity for each other generally bind slowly, tend to dissociate easily, and exhibit a large K_D. Two molecules that have high affinity for each other generally bind readily, tend to remain bound longer, and exhibit a small K_D. The K_D of two interacting molecules may be determined using methods and techniques well known in the art, e.g., surface plasmon resonance. K_D is calculated as the ratio of k_{off}/k_{on}.

As used herein, the terms "bone mineral density (BMD)" "bone density" and "bone mass" refer to a measure of the amount of bone mineral (e.g. calcium) in bone tissue. BMD may be measured by well-established clinical techniques known to one of skill in the art (e.g., by single-1 or dual-energy photon or X-ray absorptiometry). The concept of BMD relates to the mass of mineral per volume of bone, although clinically it is measured by proxy according to optical density per square centimeter of bone surface upon imaging. BMD measurement is used in clinical medicine as an indirect indicator of osteoporosis and fracture risk. In some embodiments, BMD test results are provided as a T-score, where the T-score represents the BMD of a subject compared to the ideal or peak bone mineral density of a healthy 30-year-old adult. A score of 0 indicates that the BMD is equal to the normal reference value for a healthy young adult. Differences between the measured BMD of subject and that of the reference value for a healthy young adult are measured in standard deviations units (SDs). Accordingly, a T-score of between +1 SD and -1 SD may indicate a normal BMD, a T-score of between -1 SD and -2.5 SD may indicate low bone mass (e.g., osteopenia), and a T-score lower than -2.5 SD may indicate osteoporosis or severe osteoporosis. In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)), a nucleic acid encoding such a polypeptide, or a vector containing such a nucleic acid molecule is administered to a subject in need thereof, wherein the patient has low bone mass (e.g., a

T-Score of between -1 SD and -2.5 SD). In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)), a nucleic acid encoding such a polypeptide, or a vector containing such a nucleic acid molecule is administered to a subject in need thereof, wherein the patient has osteoporosis (e.g., a T-Score of less than -2.5 SD). In some embodiments, administration of a polypeptide of the invention including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)), a nucleic acid encoding such a polypeptide, or a vector containing such a nucleic acid molecule treats the subject by increasing their BMD. In some embodiments, administration of a polypeptide of the invention including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)), a nucleic acid encoding such a polypeptide, or a vector containing such a nucleic acid molecule increases the BMD of a subject resulting in an increase in the T-Score of the subject (e.g., resulting in an increase in the T-Score of the subject of 0.1 or more, 0.2 or more, 0.3 or more, 0.4 or more, 0.5 or more, 1.0 or more, or 2.0 or more).

As used herein, the terms "bone remodeling" or "bone metabolism" refer to the process for maintaining bone strength and ion homeostasis by replacing discrete parts of old bone with newly synthesized packets of proteinaceous matrix. Bone is resorbed by osteoclasts, and is deposited by osteoblasts in a process called ossification. Osteocyte activity plays a key role in this process. Conditions that result in a decrease in bone mass, can either be caused by an increase in resorption, or a decrease in ossification. In a healthy individual, during childhood, bone formation exceeds resorption. As the aging process occurs, resorption exceeds formation. Bone resorption rates are also typically much higher in post-menopausal older women due to estrogen deficiency related to menopause.

As used herein, the terms "bone resorption" or "bone catabolic activity" refer to a process by which osteoclasts break down the tissue in bones and release the minerals, resulting in a transfer of the mineral (e.g., calcium) from bone tissue to the blood. Increased rates of bone resorption are associated with aging, including in post-menopausal women. High rates of bone resorption, or rates of bone resorption that exceed the rate of ossification, are associated with bone disorders, such as decreased bone mineral density, including osteopenia and osteoporosis. In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)), a nucleic acid encoding such a polypeptide, or a vector containing such a nucleic acid molecule is administered to a subject in need thereof to decrease done resorption in the subject (e.g., the rate of bone resorption in the subject).

As used herein, the terms "bone formation," "ossification," "osteogenesis," or "bone anabolic activity" refer to the process of forming new bone tissue by osteoblasts. In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)), a nucleic acid encoding such a polypeptide, or a vector containing such a nucleic acid molecule is administered to a subject in need thereof, to increase bone formation (e.g., increase the rate of bone formation or osteogenesis in the subject).

As used herein, the phrase "affecting myostatin, activin, and/or BMP9 signaling" means changing the binding of myostatin, activin, and/or BMP9 to their receptors, e.g., ActRIIa, ActRIIb, and BMPRII (e.g.,

ActRIIa). In some embodiments, a polypeptide including an extracellular ActRIIa variant described herein reduces or inhibits the binding of myostatin, activin, and/or BMP9 to their receptors, e.g., ActRIIa, ActRIIb, and BMPRII (e.g., ActRIIa). As described herein, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may have weak binding affinity to BMP9 (e.g., K_D of 200 pM or higher).

As used herein, the term "vascular complication" refers to a vascular disorder or any damage to the blood vessels, such as damage to the blood vessel walls. Damage to the blood vessel walls may cause an increase in vascular permeability or leakage. The term "vascular permeability or leakage" refers to the capacity of the blood vessel walls to allow the flow of small molecules, proteins, and cells in and out of blood vessels. An increase in vascular permeability or leakage may be caused by an increase in the gaps (e.g., an increase in the size and/or number of the gaps) between endothelial cells that line the blood vessel walls and/or thinning of the blood vessel walls.

As used herein, the term "polypeptide" describes a single polymer in which the monomers are amino acid residues which are covalently conjugated together through amide bonds. A polypeptide is intended to encompass any amino acid sequence, either naturally occurring, recombinant, or synthetically produced.

As used herein, the term "homodimer" refers to a molecular construct formed by two identical macromolecules, such as proteins or nucleic acids. The two identical monomers may form a homodimer by covalent bonds or non-covalent bonds. For example, an Fc domain may be a homodimer of two Fc domain monomers if the two Fc domain monomers contain the same sequence. In another example, a polypeptide described herein including an extracellular ActRIIa variant fused to an Fc domain monomer may form a homodimer through the interaction of two Fc domain monomers, which form an Fc domain in the homodimer.

As used herein, the term "heterodimer" refers to a molecular construct formed by two different macromolecules, such as proteins or nucleic acids. The two monomers may form a heterodimer by covalent bonds or non-covalent bonds. For example, a polypeptide described herein including an extracellular ActRIIa variant fused to an Fc domain monomer may form a heterodimer through the interaction of two Fc domain monomers, each fused to a different ActRIIa variant, which form an Fc domain in the heterodimer.

As used herein, the term "host cell" refers to a vehicle that includes the necessary cellular components, e.g., organelles, needed to express proteins from their corresponding nucleic acids. The nucleic acids are typically included in nucleic acid vectors that can be introduced into the host cell by conventional techniques known in the art (transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, etc.). A host cell may be a prokaryotic cell, e.g., a bacterial cell, or a eukaryotic cell, e.g., a mammalian cell (e.g., a CHO cell or a HEK293 cell).

As used herein, the term "therapeutically effective amount" refers an amount of a polypeptide, nucleic acid, or vector of the invention or a pharmaceutical composition containing a polypeptide, nucleic acid, or vector of the invention effective in achieving the desired therapeutic effect in treating a patient having a disease, such as -osteoporosis, or a condition involving bone damage, e.g., primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-

related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss. The term "therapeutically effective amount" also refers an amount of a polypeptide, nucleic acid, or vector of the invention or a pharmaceutical composition containing a polypeptide, nucleic acid, or vector of the invention effective in achieving the desired therapeutic effect in treating a patient having a disease, such as a disease or condition involving bone damage. In particular, the therapeutically effective amount of the polypeptide, nucleic acid, or vector avoids adverse side effects.

As used herein, the term "pharmaceutical composition" refers to a medicinal or pharmaceutical formulation that includes an active ingredient as well as excipients and diluents to enable the active ingredient suitable for the method of administration. The pharmaceutical composition of the present invention includes pharmaceutically acceptable components that are compatible with the polypeptide, nucleic acid, or vector. The pharmaceutical composition may be in tablet or capsule form for oral administration or in aqueous form for intravenous or subcutaneous administration.

As used herein, the term "pharmaceutically acceptable carrier or excipient" refers to an excipient or diluent in a pharmaceutical composition. The pharmaceutically acceptable carrier must be compatible with the other ingredients of the formulation and not deleterious to the recipient. In the present invention, the pharmaceutically acceptable carrier or excipient must provide adequate pharmaceutical stability to the polypeptide including an extracellular ActRIIa variant, the nucleic acid molecule(s) encoding the polypeptide, or a vector containing such nucleic acid molecule(s). The nature of the carrier or excipient differs with the mode of administration. For example, for intravenous administration, an aqueous solution carrier is generally used; for oral administration, a solid carrier is preferred.

As used herein, the term "treating and/or preventing" refers to the treatment and/or prevention of a disease, e.g., a bone disease or condition (e.g., primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), using methods and compositions of the invention. Generally, treating a bone disease or condition occurs after a subject has developed the bone disease or condition and/or is already diagnosed with the bone disease or condition. Preventing a bone disease or condition refers to steps or procedures taken when a subject is at risk of developing the bone disease or condition. The subject may show signs or mild symptoms that are judged by a physician to be indications or risk factors for developing the bone disease or condition or have a family history or genetic predisposition of developing the bone disease or condition, but has not yet developed the disease.

As used herein, the term "subject" refers to a mammal, e.g., preferably a human. Mammals include, but are not limited to, humans and domestic and farm animals, such as monkeys (e.g., a cynomolgus monkey), mice, dogs, cats, horses, and cows, etc.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a sequence alignment showing the wild-type sequences of extracellular ActRIIa and ActRIIb and the amino acid substitutions in ActRIIa variants.

DETAILED DESCRIPTION OF THE INVENTION

The invention features polypeptides that include an extracellular activin receptor type IIa (ActRIIa) variant. In some embodiments, a polypeptide of the invention includes an extracellular ActRIIa variant fused to a moiety (e.g., Fc domain monomer, a wild-type Fc domain, an Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a human serum albumin). A polypeptide including an extracellular ActRIIa variant fused to an Fc domain monomer may also form a dimer (e.g., homodimer or heterodimer) through the interaction between two Fc domain monomers. The ActRIIa variants described herein have weak binding affinity or no binding affinity to bone morphogenetic protein 9 (BMP9) compared to activins and myostatin. The invention also includes methods of treating diseases and conditions involving bone damage by increasing bone mineral density or bone formation or affecting myostatin, activin, and/or BMP9 signaling in a subject by administering to the subject a polypeptide including an extracellular ActRIIa variant described herein.

I. Extracellular activin receptor type IIa (ActRIIa) variants

Activin type II receptors are single transmembrane domain receptors that modulate signals for ligands in the transforming growth factor β (TGF-β) superfamily. Ligands in the TGF-β superfamily are involved in a host of physiological processes, such as muscle growth, vascular growth, cell differentiation, homeostasis, and osteogenesis. Examples of ligands in the TGF-β superfamily include, e.g., activin, inhibin, growth differentiation factors (GDFs) (e.g., GDF8, also known as myostatin), and bone morphogenetic proteins (BMPs) (e.g., BMP9). Activins are expressed abundantly in bone tissues and regulate bone formation by controlling both osteoblast and osteoclast functions. Activin has been reported to be upregulated in bone disease and inhibit osteoblast activity. Myostatin is also implicated in bone homeostasis through increasing osteogenesis and inhibiting osteoblast activity. These data suggest that activin receptor ligands (e.g., activin and myostatin), promote bone resorption, which could lead to diseases and conditions involving bone damage, such as primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss. Methods that reduce or inhibit this signaling could, therefore, be used in the treatment of diseases and conditions involving bone damage.

There exist two types of activin type II receptors: ActRIIa and ActRIIb. Studies have shown that BMP9 binds ActRIIb with about 300-fold higher binding affinity than ActRIIa (see, e.g., Townson et al., *J. Biol. Chem.* 287:27313, 2012). ActRIIa is known to have a longer half-life compared to ActRIIb. The present invention describes extracellular ActRIIa variants that are constructed by introducing amino acid residues of ActRIIb to ActRIIa, with the goal of imparting physiological properties conferred by ActRIIb, while also maintaining beneficial physiological and pharmacokinetic properties of ActRIIa. The optimum peptides confer significant increases in bone mineral density, while retaining longer serum half-life and low binding-affinity to BMP9, for example. The preferred ActRIIa variants also exhibit improved binding to activins and/or myostatin compared to wild-type ActRIIa, which allows them to compete with endogenous activin receptors for ligand binding and reduce or inhibit endogenous activin receptor signaling. These

variants can be used to treat disorders in which activin receptor signaling is elevated, such bone disease, leading to a reduction in bone resorption or osteoclast activity, and in increase in bone formation, bone mineral density, or bone strength. In some embodiments, amino acid substitutions may be introduced to an extracellular ActRIIa variant to reduce or remove the binding affinity of the variant to BMP9. The wild-type amino acid sequences of the extracellular portions of human ActRIIa and ActRIIb are shown below.

Human ActRIIa, extracellular portion (SEQ ID NO: 73):
GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGC
WLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS

Human ActRIIb, extracellular portion (SEQ ID NO: 74):
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWL
DDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT

Polypeptides described herein include an extracellular ActRIIa variant having at least one amino acid substitution relative to the wild-type extracellular ActRIIa having the sequence of SEQ ID NO: 73 or the extracellular ActRIIa having any one of the sequences of SEQ ID NOs: 76-96. Possible amino acid substitutions at 27 different positions may be introduced to an extracellular ActRIIa variant (Table 1). In some embodiments, an extracellular ActRIIa variant may have at least 85% (e.g., at least 85%, 87%, 90%, 92%, 95%, 97%, or greater) amino acid sequence identity to the sequence of a wild-type extracellular ActRIIa (SEQ ID NO: 73). An extracellular ActRIIa variant may have one or more (e.g., 1-27, 1-25, 1-23, 1-21, 1-19, 1-17, 1-15, 1-13, 1-11, 1-9, 1-7, 1-5, 1-3, or 1-2; e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27) amino acid substitutions relative the sequence of a wild-type extracellular ActRIIa (SEQ ID NO: 73). In some embodiments, an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having a sequence of SEQ ID NO: 1) may include amino acid substitutions at all of the 27 positions as listed in Table 1. In some embodiments, an extracellular ActRIIa variant may include amino acid substitutions at a number of positions, e.g., at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26 out of the 27 positions, as listed in Table 1.

Amino acid substitutions can worsen or improve the activity and/or binding affinity of the ActRIIa variants of the invention. To maintain polypeptide function, it is important that the lysine (K) at position X_{17} in the sequences shown in Tables 1 and 2 (SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) be retained. Substitutions at that position can lead to a loss of activity. For example, an ActRIIa variant having the sequence GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWRNISGSIEIVAKGCWLDDFNCYD RTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 150) has reduced activity in vivo, indicating that the substitution of alanine (A) for lysine (K) at X_{17} is not tolerated. ActRIIa variants of the invention, including variants in Tables 1 and 2 (e.g., SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72), therefore, retain amino acid K at position X_{17}.

The ActRIIa variants of the invention preferably have reduced, weak, or no substantial binding to BMP9. BMP9 binding is reduced in ActRIIa variants containing the amino acid sequence TEEN at positions X_{23}, X_{24}, X_{25}, and X_{26}, as well as in variants that maintain the amino acid K at position X24 and have the amino acid sequence TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}. The sequences TEEN and

TKEN can be employed interchangeably in the ActRIIa variants (e.g., the variants in Tables 1 and 2, e.g., SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) of the invention to provide reduced BMP9 binding.

The ActRIIa variants of the invention may further include a C-terminal extension (e.g., additional amino acids at the C-terminus). The C-terminal extension can add one or more additional amino acids at the C-terminus (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids) to any of the variants shown in Tables 1 and 2 (e.g., SEQ ID NOs: 1-70 (e.g., SEQ ID NOs: 6-70)). One potential C-terminal extension that can be included in the ActRIIa variants of the invention is amino acid sequence NP. For example, the sequence including the C-terminal extension is SEQ ID NO: 71 (e.g., SEQ ID NO: 69 with a C-terminal extension of NP). Another exemplary C-terminal extension that can be included in the ActRIIa variants of the invention is amino acid sequence NPVTPK (SEQ ID NO: 155). For example, the sequence including the C-terminal extension is SEQ ID NO: 72 (e.g., SEQ ID NO: 69 with a C-terminal extension of NPVTPK).

Table 1. Amino acid substitutions in an extracellular ActRIIa variant having a sequence of any one of SEQ ID NOs: 1-5

GAILGRSETQECLX$_1$X$_2$NANWX$_3$X$_4$X$_5$X$_6$TNQTGVEX$_7$CX$_8$GX$_9$X$_{10}$X$_{11}$X$_{12}$X$_{13}$X$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIV X$_{17}$X$_{18}$GCX$_{19}$X$_{20}$X$_{21}$DX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 1)

GAILGRSETQECLFX$_2$NANWX$_3$X$_4$X$_5$X$_6$TNQTGVEX$_7$CX$_8$GX$_9$KX$_{11}$X$_{12}$X$_{13}$X$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVX$_{17}$X$_{18}$GCX$_{19}$X$_{20}$X$_{21}$DX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 2)

GAILGRSETQECLFX$_2$NANWEX$_4$X$_5$RTNQTGVEX$_7$CX$_8$GX$_9$KDKRX$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVKX$_{18}$GCWL DDX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 3)

GAILGRSETQECLFX$_2$NANWEX$_4$DRTNQTGVEX$_7$CX$_8$GX$_9$KDKRX$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVKX$_{18}$GCWL DDX$_{22}$NCYDRTDCVEX$_{23}$KX$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 4)

GAILGRSETQECLFX$_2$NANWEX$_4$DRTNQTGVEPCX$_8$GX$_9$KDKRX$_{14}$HCFATWKNISGSIEIVKX$_{18}$GCWLDDI NCYDRTDCVEX$_{23}$KX$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 5)

X$_1$	F or Y	**X$_{15}$**	F or Y
X$_2$	F or Y	**X$_{16}$**	K, R, or A
X$_3$	E or A	**X$_{17}$**	K, A, Y, F, or I
X$_4$	K or L	**X$_{18}$**	Q or K
X$_5$	D or E	**X$_{19}$**	W or A
X$_6$	R or A	**X$_{20}$**	L or A
X$_7$	P or R	**X$_{21}$**	D, K, R, A, F, G, M, N, or I
X$_8$	Y or E	**X$_{22}$**	I, F, or A
X$_9$	D or E	**X$_{23}$**	K or T
X$_{10}$	K or Q	**X$_{24}$**	K or E
X$_{11}$	D or A	**X$_{25}$**	D or E
X$_{12}$	K or A	**X$_{26}$**	S or N
X13	R or A	**X$_{27}$**	E or Q
X$_{14}$	R or L		

In some embodiments of the extracellular ActRIIa variant having the sequence of SEQ ID NO: 2, X_3 is E, X_6 is R, X_{11} is D, X_{12} is K, X_{13} is R, X_{16} is K or R, X_{17} is K, X_{19} is W, X_{20} is L, X_{21} is D, and X_{22} is I or F. In some embodiments of the extracellular ActRIIa variant having the sequence of SEQ ID NO: 1 or 2, X_{17} is K. In some embodiments of the extracellular ActRIIa variant having the sequence of SEQ ID NOs: 1-3, X_{17} is K, X_{23} is T, X_{24} is E, X_{25} is E, and X_{26} is N. In some embodiments of the extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-5, X_{17} is K, X_{23} is T, X_{24} is K, X_{25} is E, and X_{26} is N.

In some embodiments, a polypeptide described herein includes an extracellular ActRIIa variant having a sequence of any one of SEQ ID NOs: 6-72 (Table 2).

Table 2. Extracellular ActRIIa variants having the sequences of SEQ ID NOs: 6-72

SEQ ID NO	Amino Acid Sequence
6	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
7	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
8	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
9	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
10	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
11	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
12	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
13	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
14	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
15	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
16	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
17	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS

SEQ ID NO	Amino Acid Sequence
18	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
19	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
20	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
21	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
22	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
23	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
24	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
25	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
26	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
27	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
28	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
29	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
30	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
31	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
32	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
33	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
34	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS

SEQ ID NO	Amino Acid Sequence
35	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
36	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
37	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
38	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
39	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
40	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
41	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
42	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
43	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCFATWKNISGSIEIV KKGC WLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
44	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
45	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
46	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
47	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
48	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDINCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
49	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
50	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS

SEQ ID NO	Amino Acid Sequence
51	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
52	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
53	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCFATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
54	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
55	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
56	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
57	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
58	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
59	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
60	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
61	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
62	GAILGRSETQECLFYNANWELDRTNQTGVEPCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
63	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETKENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
64	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDINCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
65	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWKNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
66	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCFATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
67	GAILGRSETQECLFYNANWELDRTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS

SEQ ID NO	Amino Acid Sequence
69	GAILGRSETQECLFYNANWELERTNQTGVEPCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
69	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
70	GAILGRSETQECLYYNANWELERTNQTGVERCEGEQDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS
71	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTSN P
72	GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWRNISGSIEIV KKGCWLDDFNCYDRTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTSN PVTPK

In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) has amino acid K at position X_{17}. Altering the amino acid at position X_{17} can result in reduced activity. For example, an ActRIIa variant having the sequence GAILGRSETQECLFYNANWELERTNQTGVERCEGEKDKRLHCYATWRNISGSIEIVAKGCWLDDFNCYD RTDCVETEENPQVYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 150) has reduced activity in vivo, indicating that the substitution of A for K at X_{17} is not tolerated.

In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) with the sequence TEEN at positions X_{23}, X_{24}, X_{25}, and X_{26} can have a substitution of the amino acid K for the amino acid E at position X_{24}. In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) with the sequence TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26} can have a substitution of the amino acid E for the amino acid K at position X_{24}. Polypeptides having the sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26} have reduced or weak binding to BMP9.

In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant (e.g., any one of SEQ ID NOs: 1-70 (e.g., SEQ ID NOs: 6-70)) may further include a C-terminal extension (e.g., additional amino acids at the C-terminus). In some embodiments, the C-terminal extension is amino acid sequence NP. For example, the sequence including the C-terminal extension is SEQ ID NO: 71 (e.g., SEQ ID NO: 69 with a C-terminal extension of NP). In some embodiments, the C-terminal extension is amino acid sequence NPVTPK (SEQ ID NO: 155). For example, the sequence including the C-terminal extension is SEQ ID NO: 72 (e.g., SEQ ID NO: 69 with a C-terminal extension of NPVTPK). The C-terminal extension can add one or more additional amino acids at the C-terminus (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids).

In some embodiments, a polypeptide of the invention including an extracellular ActRIIa variant may further include a moiety (e.g., Fc domain monomer, a wild-type Fc domain, an Fc domain with amino

acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a human serum albumin), which may be fused to the N- or C-terminus (e.g., C-terminus) of the extracellular ActRIIa variant by way of a linker. In some embodiments, the moiety increases the stability or improves the pharmacokinetic properties of the polypeptide. A polypeptide including an extracellular ActRIIa variant fused to an Fc domain monomer may form a dimer (e.g., homodimer or heterodimer) through the interaction between two Fc domain monomers, which combine to form an Fc domain in the dimer.

In some embodiments, an extracellular ActRIIa variant described herein does not have the sequence of any one of SEQ ID NOs: 76-96 shown in Table 3 below.

Table 3. Excluded Extracellular ActRIIa Variants.

SEQ ID NO	Amino Acid Sequence
76	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWANISGSIEIV KQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
77	GAILGRSETQECLFFNANWAKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
78	GAILGRSETQECLFFNANWEKDATNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
79	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKAKRRHCFATWKNISGSIEIV KQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
80	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDARRHCFATWKNISGSIEIV KQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
81	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKARHCFATWKNISGSIEIV KQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
82	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV AQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
83	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV YQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
84	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV FQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
85	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVI QGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
86	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCALDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
87	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWADDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS

SEQ ID NO	Amino Acid Sequence
88	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLKDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
89	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLRDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
90	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLADINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
91	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLFDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
92	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLGDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
93	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLMDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
94	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLNDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
95	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLIDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS
96	GAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIV KQGCWLDDANCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTS

Furthermore, in some embodiments, a polypeptide described herein has a serum half-life of at least 7 days in humans. The polypeptide may bind to bone morphogenetic protein 9 (BMP9) with a K_D of 200 pM or higher. The polypeptide may bind to activin A with a K_D of 10 pM or higher. In some embodiments, the polypeptide does not bind to BMP9 or activin A. In some embodiments, the polypeptide binds to activin and/or myostatin and exhibits reduced (e.g., weak) binding to BMP9. In some embodiments, the polypeptide that has reduced or weak binding to BMP9 has the sequence TEEN or TKEN at positions X_{23}, X_{24}, X_{25}, and X_{26}.

Additionally, in some embodiments, the polypeptide may bind to human BMP9 with a K_D of about 200 pM or higher (e.g., a K_D of about 200, 300, 400, 500, 600, 700, 800, or 900 pM or higher, e.g., a K_D of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, or 50 nM or higher, e.g., a K_D of between about 200 pM and about 50 nM). In some embodiments, the polypeptide does not substantially bind to human BMP9. In some embodiments, the polypeptide may bind to human activin A with a K_D of about 800 pM or less (e.g., a K_D of about 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 pM or less, e.g., a K_D of between about 800 pM and about 200 pM). In some embodiments, the polypeptide may bind to human activin B with a K_D of 800 pM or less (e.g., a K_D of about 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 pM or less, e.g., a K_D of between about 800 pM and about 200 pM) The polypeptide may also bind to growth and differentiation factor 11 (GDF-11) with a K_D of approximately 5 pM or higher (e.g., a K_D of about 5, 10, 15, 20, 25, 30,

35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, or 200 pM or higher).

II. Fc domains

In some embodiments, a polypeptide described herein may include an extracellular ActRIIa variant fused to an Fc domain monomer of an immunoglobulin or a fragment of an Fc domain to increase the serum half-life of the polypeptide. A polypeptide including an extracellular ActRIIa variant fused to an Fc domain monomer may form a dimer (e.g., homodimer or heterodimer) through the interaction between two Fc domain monomers, which form an Fc domain in the dimer. As conventionally known in the art, an Fc domain is the protein structure that is found at the C-terminus of an immunoglobulin. An Fc domain includes two Fc domain monomers that are dimerized by the interaction between the C_H3 antibody constant domains. A wild-type Fc domain forms the minimum structure that binds to an Fc receptor, e.g., FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb, FcγRIV. In some embodiments, an Fc domain may be mutated to lack effector functions, typical of a "dead" Fc domain. For example, an Fc domain may include specific amino acid substitutions that are known to minimize the interaction between the Fc domain and an Fcγ receptor. In some embodiments, an Fc domain is from an IgG1 antibody and includes amino acid substitutions L234A, L235A, and G237A. In some embodiments, an Fc domain is from an IgG1 antibody and includes amino acid substitutions D265A, K322A, and N434A. The aforementioned amino acid positions are defined according to Kabat (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The Kabat numbering of amino acid residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence. Furthermore, in some embodiments, an Fc domain does not induce any immune system-related response. For example, the Fc domain in a dimer of a polypeptide including an extracellular ActRIIa variant fused to an Fc domain monomer may be modified to reduce the interaction or binding between the Fc domain and an Fcγ receptor. The sequence of an Fc domain monomer that may be fused to an extracellular ActRIIa variant is shown below (SEQ ID NO: 97):

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGPFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

In some embodiments, an Fc domain is from an IgG1 antibody and includes amino acid substitutions L12A, L13A, and G15A, relative to the sequence of SEQ ID NO: 97. In some embodiments, an Fc domain is from an IgG1 antibody and includes amino acid substitutions D43A, K100A, and N212A, relative to the sequence of SEQ ID NO: 97. In some embodiments, an extracellular ActRIIa variant described herein (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be fused to the N- or C-terminus of an Fc domain monomer (e.g., SEQ ID NO: 97) through conventional genetic or chemical means, e.g., chemical conjugation. If desired, a linker (e.g., a spacer) can be inserted between the extracellular ActRIIa variant and the Fc domain monomer. The Fc domain monomer can be fused to the N- or C-terminus (e.g., C-terminus) of the extracellular ActRIIa variant.

In some embodiments, a polypeptide described herein may include an extracellular ActRIIa variant fused to an Fc domain. In some embodiments, the Fc domain contains one or more amino acid substitutions that reduce or inhibit Fc domain dimerization. In some embodiments, the Fc domain contains a hinge domain. The Fc domain can be of immunoglobulin antibody isotype IgG, IgE, IgM, IgA, or IgD. Additionally, the Fc domain can be an IgG subtype (e.g., IgG1, IgG2a, IgG2b, IgG3, or IgG4). The Fc domain can also be a non-naturally occurring Fc domain, e.g., a recombinant Fc domain.

Methods of engineering Fc domains that have reduced dimerization are known in the art. In some embodiments, one or more amino acids with large side-chains (e.g., tyrosine or tryptophan) may be introduced to the C_H3-C_H3 dimer interface to hinder dimer formation due to steric clash. In other embodiments, one or more amino acids with small side-chains (e.g., alanine, valine, or threonine) may be introduced to the C_H3-C_H3 dimer interface to remove favorable interactions. Methods of introducing amino acids with large or small side-chains in the C_H3 domain are described in, e.g., Ying et al. (*J Biol Chem.* 287:19399-19408, 2012), U.S. Patent Publication No. 2006/0074225, U.S. Patent Nos. 8,216,805 and 5,731,168, Ridgway et al. (*Protein Eng.* 9:617-612, 1996), Atwell et al. (*J Mol Biol.* 270:26-35, 1997), and Merchant et al. (*Nat Biotechnol.* 16:677-681, 1998), all of which are incorporated herein by reference in their entireties.

In yet other embodiments, one or more amino acid residues in the C_H3 domain that make up the C_H3-C_H3 interface between two Fc domains are replaced with positively-charged amino acid residues (e.g., lysine, arginine, or histidine) or negatively-charged amino acid residues (e.g., aspartic acid or glutamic acid) such that the interaction becomes electrostatically unfavorable depending on the specific charged amino acids introduced. Methods of introducing charged amino acids in the C_H3 domain to disfavor or prevent dimer formation are described in, e.g., Ying et al. (*J Biol Chem.* 287:19399-19408, 2012), U.S. Patent Publication Nos. 2006/0074225, 2012/0244578, and 2014/0024111, all of which are incorporated herein by reference in their entireties.

In some embodiments of the invention, an Fc domain includes one or more of the following amino acid substitutions:T366W, T366Y, T394W, F405W, Y349T, Y349E, Y349V, L351T, L351H, L351N, L352K, P353S, S354D, D356K, D356R, D356S, E357K, E357R, E357Q, S364A, T366E, L368T, L368Y, L368E, K370E, K370D, K370Q, K392E, K392D, T394N, P395N, P396T, V397T, V397Q, L398T, D399K, D399R, D399N, F405T, F405H, F405R, Y407T, Y407H, Y407I, K409E, K409D, K409T, and K409I, relative to the sequence of human IgG1. In one particular embodiment, an Fc domain includes the amino acid substitution T366W, relative to the sequence of human IgG1. The sequence of wild-type Fc domain is shown in SEQ ID NO: 151.

III. Albumin-binding peptide

In some embodiments, a polypeptide described herein may include an extracellular ActRIIa variant fused to a serum protein-binding peptide. Binding to serum protein peptides can improve the pharmacokinetics of protein pharmaceuticals.

As one example, albumin-binding peptides that can be used in the methods and compositions described here are generally known in the art. In one embodiment, the albumin binding peptide includes the sequence DICLPRWGCLW (SEQ ID NO: 152).

In the present invention, albumin-binding peptides may be joined to the N- or C-terminus (e.g., C-terminus) of an extracellular ActRIIa variant described herein (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) to increase the serum half-life of the extracellular ActRIIa variant. In some embodiments, an albumin-binding peptide is joined, either directly or through a linker, to the N- or C-terminus of an extracellular ActRIIa variant.

In some embodiments, an extracellular ActRIIa variant described herein (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be fused to the N- or C-terminus of albumin-binding peptide (e.g., SEQ ID NO: 152) through conventional genetic or chemical means, e.g., chemical conjugation. If desired, a linker (e.g., a spacer) can be inserted between the extracellular ActRIIa variant and the albumin-binding peptide. Without being bound to a theory, it is expected that inclusion of an albumin-binding peptide in an extracellular ActRIIa variant described herein may lead to prolonged retention of the therapeutic protein through its binding to serum albumin.

IV. Fibronectin domain

In some embodiments, a polypeptide described herein may include an extracellular ActRIIa variant fused to fibronectin domains. Binding to fibronectin domains can improve the pharmacokinetics of protein pharmaceuticals.

Fibronectin domain is a high molecular weight glycoprotein of the extracellular matrix, or a fragment thereof, that binds to, e.g., membrane-spanning receptor proteins such as integrins and extracellular matrix components such as collagens and fibrins. In some embodiments of the present invention, a fibronectin domain is joined to the N- or C-terminus (e.g., C-terminus) of an extracellular ActRIIa variant described herein (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) to increase the serum half-life of the extracellular ActRIIa variant. A fibronectin domain can be joined, either directly or through a linker, to the N- or C-terminus of an extracellular ActRIIa variant.

As one example, fibronectin domains that can be used in the methods and compositions described here are generally known in the art. In one embodiment, the fibronectin domain is a fibronectin type III domain (SEQ ID NO: 153) having amino acids 610-702 of the sequence of UniProt ID NO: P02751. In another embodiment, the fibronectin domain is an adnectin protein.

In some embodiments, an extracellular ActRIIa variant described herein (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be fused to the N- or C-terminus of a fibronectin domain (e.g., SEQ ID NO: 153) through conventional genetic or chemical means, e.g., chemical conjugation. If desired, a linker (e.g., a spacer) can be inserted between the extracellular ActRIIa variant and the fibronectin domain. Without being bound to a theory, it is expected that inclusion of a fibronectin domain in an extracellular ActRIIa variant described herein may lead to prolonged retention of the therapeutic protein through its binding to integrins and extracellular matrix components such as collagens and fibrins.

V. Serum albumin

In some embodiments, a polypeptide described herein may include an extracellular ActRIIa variant fused to serum albumin. Binding to serum albumins can improve the pharmacokinetics of protein pharmaceuticals.

Serum albumin is a globular protein that is the most abundant blood protein in mammals. Serum albumin is produced in the liver and constitutes about half of the blood serum proteins. It is monomeric and soluble in the blood. Some of the most crucial functions of serum albumin include transporting hormones, fatty acids, and other proteins in the body, buffering pH, and maintaining osmotic pressure needed for proper distribution of bodily fluids between blood vessels and body tissues. In preferred embodiments, serum albumin is human serum albumin. In some embodiments of the present invention, a human serum albumin is joined to the N- or C-terminus (e.g., C-terminus) of an extracellular ActRIIa variant described herein (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) to increase the serum half-life of the extracellular ActRIIa variant. A human serum albumin can be joined, either directly or through a linker, to the N- or C-terminus of an extracellular ActRIIa variant.

As one example, serum albumins that can be used in the methods and compositions described herein are generally known in the art. In one embodiment, the serum albumin includes the sequence of UniProt ID NO: P02768 (SEQ ID NO: 154).

In some embodiments, an extracellular ActRIIa variant described herein (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be fused to the N- or C-terminus of a human serum albumin (e.g., SEQ ID NO: 154) through conventional genetic or chemical means, e.g., chemical conjugation. If desired, a linker (e.g., a spacer) can be inserted between the extracellular ActRIIa variant and the serum albumin. Without being bound to a theory, it is expected that inclusion of a serum albumin in an extracellular ActRIIa variant described herein may lead to prolonged retention of the therapeutic protein.

VI. Linkers

A polypeptide described herein may include an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having a sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) fused to a moiety by way of a linker. In some embodiments, the moiety increases stability of the polypeptide. Exemplary moieties include an Fc domain monomer, a wild-type Fc domain, an Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a human serum albumin. In the present invention, a linker between a moiety (e.g., an Fc domain monomer (e.g., the sequence of SEQ ID NO: 97), a wild-type Fc domain (e.g., SEQ ID NO: 151), an Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide (e.g., SEQ ID NO: 152), a fibronectin domain (e.g., SEQ ID NO: 153), or a human serum albumin (e.g., SEQ ID NO: 154)) and an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)), can be an amino acid spacer including 1-200 amino acids. Suitable peptide spacers are known in the art, and include, for example, peptide linkers containing flexible amino acid residues such as glycine, alanine, and serine. In some embodiments, a spacer can contain motifs, e.g., multiple or repeating

motifs, of GA, GS, GG, GGA, GGS, GGG, GGGA (SEQ ID NO: 98), GGGS (SEQ ID NO: 99), GGGG (SEQ ID NO: 100), GGGGA (SEQ ID NO: 101), GGGGS (SEQ ID NO: 102), GGGGG (SEQ ID NO: 103), GGAG (SEQ ID NO: 104), GGSG (SEQ ID NO: 105), AGGG (SEQ ID NO: 106), or SGGG (SEQ ID NO: 107). In some embodiments, a spacer can contain 2 to 12 amino acids including motifs of GA or GS, e.g., GA, GS, GAGA (SEQ ID NO: 108), GSGS (SEQ ID NO: 109), GAGAGA (SEQ ID NO: 110), GSGSGS (SEQ ID NO: 111), GAGAGAGA (SEQ ID NO: 112), GSGSGSGS (SEQ ID NO: 113), GAGAGAGAGA (SEQ ID NO: 114), GSGSGSGSGS (SEQ ID NO: 115), GAGAGAGAGAGA (SEQ ID NO: 116), and GSGSGSGSGSGS (SEQ ID NO: 117). In some embodiments, a spacer can contain 3 to 12 amino acids including motifs of GGA or GGS, e.g., GGA, GGS, GGAGGA (SEQ ID NO: 118), GGSGGS (SEQ ID NO: 119), GGAGGAGGA (SEQ ID NO: 120), GGSGGSGGS (SEQ ID NO: 121), GGAGGAGGAGGA (SEQ ID NO: 122), and GGSGGSGGSGGS (SEQ ID NO: 123). In yet some embodiments, a spacer can contain 4 to 12 amino acids including motifs of GGAG (SEQ ID NO: 104), GGSG (SEQ ID NO: 105), e.g., GGAG (SEQ ID NO: 104), GGSG (SEQ ID NO: 105), GGAGGGAG (SEQ ID NO: 124), GGSGGGSG (SEQ ID NO: 125), GGAGGGAGGGAG (SEQ ID NO: 126), and GGSGGGSGGGSG (SEQ ID NO: 127). In some embodiments, a spacer can contain motifs of GGGGA (SEQ ID NO: 101) or GGGGS (SEQ ID NO: 102), e.g., GGGGAGGGGAGGGGA (SEQ ID NO: 128) and GGGGSGGGGSGGGGS (SEQ ID NO: 129). In some embodiments of the invention, an amino acid spacer between a moiety (e.g., an Fc domain monomer, a wild-type Fc domain, an Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a serum albumin) and an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be GGG, GGGA (SEQ ID NO: 98), GGGG (SEQ ID NO: 100), GGGAG (SEQ ID NO: 130), GGGAGG (SEQ ID NO: 131), or GGGAGGG (SEQ ID NO: 132).

In some embodiments, a spacer can also contain amino acids other than glycine, alanine, and serine, e.g., AAAL (SEQ ID NO: 133), AAAK (SEQ ID NO: 134), AAAR (SEQ ID NO: 135), EGKSSGSGSESKST (SEQ ID NO: 136), GSAGSAAGSGEF (SEQ ID NO: 137), AEAAAKEAAAKA (SEQ ID NO: 138), KESGSVSSEQLAQFRSLD (SEQ ID NO: 139), GENLYFQSGG (SEQ ID NO: 140), SACYCELS (SEQ ID NO: 141), RSIAT (SEQ ID NO: 142), RPACKIPNDLKQKVMNH (SEQ ID NO: 143), GGSAGGSGSGSSGGSSGASGTGTAGGTGSGSGTGSG (SEQ ID NO: 144), AAANSSIDLISVPVDSR (SEQ ID NO: 145), or GGSGGGSEGGGSEGGGSEGGGSEGGGSEGGGSGGGS (SEQ ID NO: 146). In some embodiments, a spacer can contain motifs, e.g., multiple or repeating motifs, of EAAAK (SEQ ID NO: 147). In some embodiments, a spacer can contain motifs, e.g., multiple or repeating motifs, of proline-rich sequences such as $(XP)_n$, in which X may be any amino acid (e.g., A, K, or E) and n is from 1-5, and PAPAP(SEQ ID NO: 148).

The length of the peptide spacer and the amino acids used can be adjusted depending on the two protein involved and the degree of flexibility desired in the final protein fusion polypeptide. The length of the spacer can be adjusted to ensure proper protein folding and avoid aggregate formation.

VII. Vectors, host cells, and protein production

The polypeptides of the invention can be produced from a host cell. A host cell refers to a vehicle that includes the necessary cellular components, e.g., organelles, needed to express the polypeptides

and fusion polypeptides described herein from their corresponding nucleic acids. The nucleic acids may be included in nucleic acid vectors that can be introduced into the host cell by conventional techniques known in the art (e.g., transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, infection, or the like). The choice of nucleic acid vectors depends in part on the host cells to be used. Generally, preferred host cells are of either eukaryotic (e.g., mammalian) or prokaryotic (e.g., bacterial) origin.

Nucleic acid vector construction and host cells

A nucleic acid sequence encoding the amino acid sequence of a polypeptide of the invention may be prepared by a variety of methods known in the art. These methods include, but are not limited to, oligonucleotide-mediated (or site-directed) mutagenesis and PCR mutagenesis. A nucleic acid molecule encoding a polypeptide of the invention may be obtained using standard techniques, e.g., gene synthesis. Alternatively, a nucleic acid molecule encoding a wild-type extracellular ActRIIa may be mutated to include specific amino acid substitutions using standard techniques in the art, e.g., QuikChange™ mutagenesis. Nucleic acid molecules can be synthesized using a nucleotide synthesizer or PCR techniques.

A nucleic acid sequence encoding a polypeptide of the invention may be inserted into a vector capable of replicating and expressing the nucleic acid molecule in prokaryotic or eukaryotic host cells. Many vectors are available in the art and can be used for the purpose of the invention. Each vector may include various components that may be adjusted and optimized for compatibility with the particular host cell. For example, the vector components may include, but are not limited to, an origin of replication, a selection marker gene, a promoter, a ribosome binding site, a signal sequence, the nucleic acid sequence encoding protein of interest, and a transcription termination sequence.

In some embodiments, mammalian cells may be used as host cells for the invention. Examples of mammalian cell types include, but are not limited to, human embryonic kidney (HEK) (e.g., HEK293, HEK 293F), Chinese hamster ovary (CHO), HeLa, COS, PC3, Vero, MC3T3, NS0, Sp2/0, VERY, BHK, MDCK, W138, BT483, Hs578T, HTB2, BT20, T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7O3O, and HsS78Bst cells. In some embodiments, *E. coli* cells may also be used as host cells for the invention. Examples of *E. coli* strains include, but are not limited to, *E. coli* 294 (ATCC® 31,446), *E. coli* λ 1776 (ATCC® 31,537, *E. coli* BL21 (DE3) (ATCC® BAA-1025), and *E. coli* RV308 (ATCC® 31,608). Different host cells have characteristic and specific mechanisms for the posttranslational processing and modification of protein products (e.g., glycosylation). Appropriate cell lines or host systems may be chosen to ensure the correct modification and processing of the polypeptide expressed. The above-described expression vectors may be introduced into appropriate host cells using conventional techniques in the art, e.g., transformation, transfection, electroporation, calcium phosphate precipitation, and direct microinjection. Once the vectors are introduced into host cells for protein production, host cells are cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Methods for expression of therapeutic proteins are known in the art, see, for example, Paulina Balbas, Argelia Lorence (eds.) *Recombinant Gene Expression: Reviews and Protocols (Methods in Molecular Biology)*, Humana Press; 2nd ed. 2004 and Vladimir Voynov and Justin

A. Caravella (eds.) *Therapeutic Proteins: Methods and Protocols (Methods in Molecular Biology)* Humana Press; 2nd ed. 2012.

Protein production, recovery, and purification

Host cells used to produce the polypeptides of the invention may be grown in media known in the art and suitable for culturing of the selected host cells. Examples of suitable media for mammalian host cells include Minimal Essential Medium (MEM), Dulbecco's Modified Eagle's Medium (DMEM), Expi293™ Expression Medium, DMEM with supplemented fetal bovine serum (FBS), and RPMI-1640. Examples of suitable media for bacterial host cells include Luria broth (LB) plus necessary supplements, such as a selection agent, e.g., ampicillin. Host cells are cultured at suitable temperatures, such as from about 20 °C to about 39 °C, e.g., from 25 °C to about 37 °C, preferably 37 °C, and CO_2 levels, such as 5 to 10%. The pH of the medium is generally from about 6.8 to 7.4, e.g., 7.0, depending mainly on the host organism. If an inducible promoter is used in the expression vector of the invention, protein expression is induced under conditions suitable for the activation of the promoter.

In some embodiments, depending on the expression vector and the host cells used, the expressed protein may be secreted from the host cells (e.g., mammalian host cells) into the cell culture media. Protein recovery may involve filtering the cell culture media to remove cell debris. The proteins may be further purified. A polypeptide of the invention may be purified by any method known in the art of protein purification, for example, by chromatography (e.g., ion exchange, affinity, and size-exclusion column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. For example, the protein can be isolated and purified by appropriately selecting and combining affinity columns such as Protein A column (e.g., POROS Protein A chromatography) with chromatography columns (e.g., POROS HS-50 cation exchange chromatography), filtration, ultra filtration, salting-out and dialysis procedures.

In other embodiments, host cells may be disrupted, e.g., by osmotic shock, sonication, or lysis, to recover the expressed protein. Once the cells are disrupted, cell debris may be removed by centrifugation or filtration. In some instances, a polypeptide can be conjugated to marker sequences, such as a peptide to facilitate purification. An example of a marker amino acid sequence is a hexa-histidine peptide (His-tag), which binds to nickel-functionalized agarose affinity column with micromolar affinity. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin "HA" tag, which corresponds to an epitope derived from influenza hemagglutinin protein (Wilson et al., *Cell* 37:767, 1984).

Alternatively, the polypeptides of the invention can be produced by the cells of a subject (e.g., a human), e.g., in the context of gene therapy, by administrating a vector (such as a viral vector (e.g., a retroviral vector, adenoviral vector, poxviral vector (e.g., vaccinia viral vector, such as Modified Vaccinia Ankara (MVA)), adeno-associated viral vector, and alphaviral vector)) containing a nucleic acid molecule encoding the polypeptide of the invention. The vector, once inside a cell of the subject (e.g., by transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, infection, etc.) will promote expression of the polypeptide, which is then secreted from the cell. If treatment of a disease or disorder is the desired outcome, no further action may be required. If collection

of the protein is desired, blood may be collected from the subject and the protein purified from the blood by methods known in the art.

VIII. Pharmaceutical compositions and preparations

The invention features pharmaceutical compositions that include the polypeptides described herein (e.g., a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)). In some embodiments, a pharmaceutical composition of the invention includes a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-70 (e.g., SEQ ID NOs: 6-70)) with a C-terminal extension (e.g., 1, 2, 3, 4, 5, 6, or more additional amino acids) as the therapeutic protein. In some embodiments, a pharmaceutical composition of the invention includes a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) fused to a moiety (e.g., a Fc domain monomer, or a dimer thereof, a wild-type Fc domain, an Fc domain with amino acid substitutions (e.g., one or more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a serum albumin) as the therapeutic protein. In some embodiments, a pharmaceutical composition of the invention including a polypeptide of the invention may be used in combination with other agents (e.g., therapeutic biologics and/or small molecules) or compositions in a therapy. In addition to a therapeutically effective amount of the polypeptide, the pharmaceutical composition may include one or more pharmaceutically acceptable carriers or excipients, which can be formulated by methods known to those skilled in the art. In some embodiments, a pharmaceutical composition of the invention includes a nucleic acid molecule (DNA or RNA, e.g., mRNA) encoding a polypeptide of the invention, or a vector containing such a nucleic acid molecule.

Acceptable carriers and excipients in the pharmaceutical compositions are nontoxic to recipients at the dosages and concentrations employed. Acceptable carriers and excipients may include buffers such as phosphate, citrate, HEPES, and TAE, antioxidants such as ascorbic acid and methionine, preservatives such as hexamethonium chloride, octadecyldimethylbenzyl ammonium chloride, resorcinol, and benzalkonium chloride, proteins such as human serum albumin, gelatin, dextran, and immunoglobulins, hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, histidine, and lysine, and carbohydrates such as glucose, mannose, sucrose, and sorbitol. Pharmaceutical compositions of the invention can be administered parenterally in the form of an injectable formulation. Pharmaceutical compositions for injection can be formulated using a sterile solution or any pharmaceutically acceptable liquid as a vehicle. Pharmaceutically acceptable vehicles include, but are not limited to, sterile water, physiological saline, and cell culture media (e.g., Dulbecco's Modified Eagle Medium (DMEM), α-Modified Eagles Medium (α-MEM), F-12 medium). Formulation methods are known in the art, see e.g., Banga (ed.) *Therapeutic Peptides and Proteins: Formulation, Processing and Delivery Systems* (3rd ed.) Taylor & Francis Group, CRC Press (2015).

The pharmaceutical compositions of the invention may be prepared in microcapsules, such as hydroxylmethylcellulose or gelatin-microcapsule and poly-(methylmethacrylate) microcapsule. The pharmaceutical compositions of the invention may also be prepared in other drug delivery systems such as liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules. Such

techniques are described in Remington: The Science and Practice of Pharmacy 22th edition (2012). The pharmaceutical compositions to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

The pharmaceutical compositions of the invention may also be prepared as a sustained-release formulation. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptides of the invention. Examples of sustained release matrices include polyesters, hydrogels, polyactides, copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as LUPRON DEPOT™, and poly-D-(-)-3-hydroxybutyric acid. Some sustained-release formulations enable release of molecules over a few months, e.g., one to six months, while other formulations release pharmaceutical compositions of the invention for shorter time periods, e.g., days to weeks.

The pharmaceutical composition may be formed in a unit dose form as needed. The amount of active component, e.g., a polypeptide of the invention, included in the pharmaceutical preparations is such that a suitable dose within the designated range is provided (e.g., a dose within the range of 0.01-100 mg/kg of body weight).

The pharmaceutical composition for gene therapy can be in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. If hydrodynamic injection is used as the delivery method, the pharmaceutical composition containing a nucleic acid molecule encoding a polypeptide described herein or a vector (e.g., a viral vector) containing the nucleic acid molecule is delivered rapidly in a large fluid volume intravenously. Vectors that may be used as in vivo gene delivery vehicle include, but are not limited to, retroviral vectors, adenoviral vectors, poxviral vectors (e.g., vaccinia viral vectors, such as Modified Vaccinia Ankara), adeno-associated viral vectors, and alphaviral vectors.

IX. Routes, dosage, and administration

Pharmaceutical compositions that include the polypeptides of the invention as the therapeutic proteins may be formulated for, e.g., intravenous administration, parenteral administration, subcutaneous administration, intramuscular administration, intra-arterial administration, intrathecal administration, or intraperitoneal administration. The pharmaceutical composition may also be formulated for, or administered via, oral, nasal, spray, aerosol, rectal, or vaginal administration. For injectable formulations, various effective pharmaceutical carriers are known in the art. See, e.g., ASHP Handbook on Injectable Drugs, Toissel, 18th ed. (2014).

In some embodiments, a pharmaceutical composition that includes a nucleic acid molecule encoding a polypeptide of the invention or a vector containing such nucleic acid molecule may be administered by way of gene delivery. Methods of gene delivery are well-known to one of skill in the art. Vectors that may be used for in vivo gene delivery and expression include, but are not limited to, retroviral vectors, adenoviral vectors, poxviral vectors (e.g., vaccinia viral vectors, such as Modified Vaccinia Ankara (MVA)), adeno-associated viral vectors, and alphaviral vectors. In some embodiments, mRNA molecules encoding polypeptides of the invention may be administered directly to a subject.

In some embodiments of the present invention, nucleic acid molecules encoding a polypeptide described herein or vectors containing such nucleic acid molecules may be administered using a hydrodynamic injection platform. In the hydrodynamic injection method, a nucleic acid molecule encoding

a polypeptide described herein is put under the control of a strong promoter in an engineered plasmid (e.g., a viral plasmid). The plasmid is often delivered rapidly in a large fluid volume intravenously. Hydrodynamic injection uses controlled hydrodynamic pressure in veins to enhance cell permeability such that the elevated pressure from the rapid injection of the large fluid volume results in fluid and plasmid extravasation from the vein. The expression of the nucleic acid molecule is driven primarily by the liver. In mice, hydrodynamic injection is often performed by injection of the plasmid into the tail vein. In certain embodiments, mRNA molecules encoding a polypeptide described herein may be administered using hydrodynamic injection.

The dosage of the pharmaceutical compositions of the invention depends on factors including the route of administration, the disease to be treated, and physical characteristics, e.g., age, weight, general health, of the subject. A pharmaceutical composition of the invention may include a dosage of a polypeptide of the invention ranging from 0.01 to 500 mg/kg (e.g., 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 mg/kg) and, in a more specific embodiment, about 0.1 to about 30 mg/kg and, in a more specific embodiment, about 0.3 to about 30 mg/kg. The dosage may be adapted by the physician in accordance with conventional factors such as the extent of the disease and different parameters of the subject.

The pharmaceutical compositions are administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective to result in an improvement or remediation of the symptoms. The pharmaceutical compositions are administered in a variety of dosage forms, e.g., intravenous dosage forms, subcutaneous dosage forms, and oral dosage forms (e.g., ingestible solutions, drug release capsules). Generally, therapeutic proteins are dosed at 0.1-100 mg/kg, e.g., 1-50 mg/kg. Pharmaceutical compositions that include a polypeptide of the invention may be administered to a subject in need thereof, for example, one or more times (e.g., 1-10 times or more) daily, weekly, biweekly, monthly, bimonthly, quarterly, biannually, annually, or as medically necessary. In some embodiments, pharmaceutical compositions that include a polypeptide of the invention may be administered to a subject in need thereof weekly, biweekly, monthly, bimonthly, or quarterly. Dosages may be provided in either a single or multiple dosage regimens. The timing between administrations may decrease as the medical condition improves or increase as the health of the patient declines.

X. Methods of treatment

The invention is based on the discovery that substituting amino acids from the extracellular portion of ActRIIb into the extracellular portion ActRIIa yields ActRIIa variants with improved properties. The ActRIIa variants generated by introducing residues from ActRIIb into ActRIIa retain the beneficial properties of ActRIIa, such as longer serum half-life and low binding affinity to BMP9, and gain some of the beneficial properties of ActRIIb, such as increased binding to activins A and B (see Table 4). These ActRIIa variant properties produce a polypeptide that can be used therapeutically to compete with endogenous activin receptors for ligand binding. As the ActRIIa variants contain the extracellular portion of the receptor, they are soluble and able to bind to and sequester ligands (e.g., activins A and B, myostatin, GDF11) without activating intracellular signaling pathways. Therefore, the extracellular ActRIIa variants can be used to treat diseases or conditions in which elevated activin signaling has been implicated (e.g., associated with increased expression of activin receptors or activin receptor ligands).

For example, activin has been found to be upregulated in bone disease and is known to inhibit osteoblast activity, suggesting that increased activin levels contribute to bone disease. It follows that treatment with a therapeutic agent that binds to activin and reduces its interaction with endogenous receptors could be used to increase bone mineral density and treat subjects with diseases or conditions involving bone damage.

The invention provides compositions and methods of treatment that may be used to increase bone mineral density, increase bone formation, or reduce bone resorption in a subject in need thereof. In some embodiments, the subject may have a disease that results in bone damage (e.g., osteoporosis or osteopenia). In some embodiments, the methods described herein are directed to affecting myostatin, activin, and/or BMP9 signaling in a subject having a disease or condition involving bone damage. In some embodiments, a polypeptide including an extracellular ActRIIa variant described herein reduces or inhibits the binding of myostatin, activin, and/or BMP9 to their receptors, e.g., ActRIIa, ActRIIb, and BMPRII (e.g., ActRIIa). In some embodiments, affecting myostatin, activin, and/or BMP9 signaling (e.g., reducing or inhibiting the binding of myostatin, activin, and/or BMP9 to their receptors, e.g., ActRIIa, ActRIIb, and BMPRII (e.g., ActRIIa)) results in an increase in the subject's bone mineral density or bone formation, or a decrease in the subject's bone resorption.

In some embodiments, the polypeptides described herein (e.g., a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be administered to a subject to increase bone mineral density, to increase bone formation, to decrease bone resorption, or to affect myostatin, activin, and/or BMP9 signaling in the subject. In some embodiments, the methods described herein increase bone mineral density of the subject. In some embodiments, the methods described herein do not cause any vascular complications in the subject, such as increased vascular permeability or leakage. In some embodiments of the methods described herein, the subject has a disease or condition involving bone damage (e.g., primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss).

The invention also includes methods of treating a subject having primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss by administering to the subject a polypeptide described herein (e.g., a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)). In some embodiments, the primary osteoporosis is age-related or hormone-related osteoporosis (e.g., related to a decline in estrogen). In some embodiments, the secondary osteoporosis is immobilization-induced or glucocorticoid-induced osteoporosis. In some embodiments, the bone cancer is multiple myeloma or the cancer metastasis-related bone loss is caused by multiple myeloma. In some embodiments, the treatment-related bone loss occurs due to treatment with FGF-21 or GLP-1, treatment with an FGF-21 or GLP-1 containing therapeutic, or treatment of Type-2 diabetes and/or obesity, or due to cancer therapy (e.g., chemotherapy or radiation). In some

embodiments, the diet-related bone loss is rickets (e.g., vitamin D deficiency). In some embodiments, the low-gravity related bone loss is lack of load-related bone loss.

In some embodiments, the polypeptides described herein (e.g., a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be used to prevent the development of a disease or condition involving bone damage (e.g., primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, osteopetrosis, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss) and/or to treat patients already diagnosed with a disease or condition involving bone damage. Patients who are likely to develop a disease or condition involving bone damage, e.g., individuals with genetic predisposition, family history of bone damage, or low bone mass, may be administered the polypeptides described herein (e.g., a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) prophylactically, such that the extracellular ActRIIa polypeptides may prevent or delay the development of bone damage.

In some embodiments, the polypeptides described herein (e.g., a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) may be administered to a subject to prevent the development of and/or treat patients with a disease or condition involving bone damage (e.g., primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss), or to affect myostatin, activin, and/or BMP9 signaling in the subject (e.g., to reduce or inhibit the binding of activin, myostatin, and/or BMP9 to their receptors). In some embodiments, the methods described herein increase bone mineral density (e.g., increase bone mass). In some embodiments, the methods described herein reduce bone resorption (e.g., reduce bone catabolic activity). In some embodiments, the methods described herein increase bone formation (e.g., increase bone anabolic activity or increase osteogenesis). In some embodiments, the methods described herein increase osteoblast activity or osteoblastogenesis. In some embodiments, the methods described herein decrease osteoclast activity or osteoclastogenesis. In some embodiments, the methods described herein reduce or inhibit the binding of activin and/or myostatin to their receptors. In some embodiments, the methods increase bone formation, increase bone mineral density, or decrease bone resorption of cortical or trabecular bone.

In any of the methods described herein, a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-71 (e.g., SEQ ID NOs: 6-71)) that further includes a C-terminal extension of one or more amino acids (e.g., 1, 2, 3, 4, 5, 6, or more amino acids) may be used as the therapeutic protein. In any of the methods described herein, a dimer (e.g., homodimer or heterodimer) of a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) fused to an Fc domain monomer may be used as the therapeutic protein. In any of the methods described herein, a polypeptide including an extracellular ActRIIa variant (e.g., an extracellular ActRIIa

variant having the sequence of any one of SEQ ID NOs: 1-72 (e.g., SEQ ID NOs: 6-72)) fused to a moiety (e.g., a wild-type Fc domain, an Fc domain with amino acid substitutions (e.g., one more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a serum albumin) may be used as the therapeutic protein. Nucleic acids encoding the polypeptides described herein, or vectors containing said nucleic acids can also be administered according to any of the methods described herein. In any of the methods described herein, the polypeptide, nucleic acid, or vector can be administered as part of a pharmaceutical composition.

EXAMPLES

Example 1 – Evaluation of ActRIIa variants binding affinity by surface plasmon resonance (SPR)

The Biacore 3000 was used to measure the kinetics of the interactions between the ActRIIa variants and the ligands Activin A, Activin B, growth differentiation factor 11 (GDF11), and BMP-9. ActRIIa variants were transiently expressed in HEK293 cells and purified from the conditioned media using Protein-A Sepharose chromatography. The ActRIIa variants were immobilized on the chip (CM4 or CM5) with capture antibodies (anti-mouse from GEGE) in flow cells 2-4 to ensure proper orientation. Flow cell 1 was used as a reference cell to subtract any nonspecific binding and bulk effects. HBS-EP+ buffer from GE Healthcare™ was used as a running buffer. Each ligand was run in a concentration series at 40 µl/min to avoid mass transport effects. The data was analyzed using Scrubber2 by BioLogic™ Software to calculate the K_D of each interaction (Table 4).

Table 4: Comparison of ActRIIa variant binding affinity (K_D) to various ligands

	Activin A (K_D)	**Activin B (K_D)**	**GDF-11 (K_D)**	**BMP-9 (K_D)**
Vehicle	**N/A**	**N/A**	**N/A**	**N/A**
ActRIIa (SEQ ID NO: 73)	1 nM	373 pM	81 pM	25 nM
ActRIIb (SEQ ID NO: 74)	63 pM	23 pM	115 pM	278 pM
ActRIIa/b variant (SEQ ID NO: 69)	542 pM	103 pM	186 pM	4 nM
ActRIIb/a variant (SEQ ID NO: 149)	No Binding	No Binding	No Binding	No Binding
ActRIIa/bΔ9 variant (SEQ ID NO: 58)	213 pM	12.3 pM	115 pM	10 nM
ActRIIa/bΔ9 min variant (SEQ ID NO: 6)	310 pM	88 pM	114 pM	17 nM
ActRIIa/b+ variant (SEQ ID NO: 150)	242 pM	282 pM	No dissociation	26 nM
ActRIIa/bΔ9m2 variant	170 pM	104 pM	222 pM	13-18 nM

	Activin A (K_D)	Activin B (K_D)	GDF-11 (K_D)	BMP-9 (K_D)
(SEQ ID NO: 38)				
ActRIIa/bΔ9m3 variant (SEQ ID NO: 41)	71 pM	72.5 pM	117 pM	1.2 nM
ActRIIa/bΔ9m4 variant (SEQ ID NO: 44)	375 pM	254 pM	394 pM	14-20 nM
ActRIIa/bmax1 variant (SEQ ID NO: 70)	232 pM	97 pM	236 pM	5.6 nM
ActRIIa/bmax2 variant (SEQ ID NO: 71)	135 pM	39 pM	113 pM	5 nM
ActRIIa/bmax3 variant (SEQ ID NO: 72)	89 pM	43 pM	214 pM	3.3 nM

Example 2 - Effect of extracellular ActRIIa variants on bone mineral density

Adult male C57/BL6 mice receive either a sham- (SHAM) or castration-surgery (ORX). Both surgery groups are allowed to recover for 14 days post-surgery. All animals are housed in conventional cages with free access to food (regular chow) and water. SHAM and ORX animals are then assigned to either a vehicle-treated group (VEH) or ActRII variant-treated group and receive bi-weekly systemic intraperitoneal administration of vehicle or ActRII variant (10 mg/kg) for 71 d. Body weights are measured twice per week at the time of treatment. Body composition is analyzed at study day 0 then at days 14, 28, 47, and 71 after treatment initiation using the MiniSpec LF50 NMR Analyzer. At study termination date, tissues of interest (muscles, fat depots, and tibias) are surgically removed, weighed, and properly stored for further analysis. At this time, the ORX animals are also examined to confirm complete removal of testes. Cortical morphometry and trabecular structure of the various bones are also evaluated after the experiment termination using micro-computed tomography.

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

All publications, patents, and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Other embodiments are within the following claims.

CLAIMS

What is claimed is:

1. A polypeptide comprising an extracellular activin receptor type IIa (ActRIIa) variant, the variant having a sequence of GAILGRSETQECLX$_1$X$_2$NANWX$_3$X$_4$X$_5$X$_6$TNQTGVEX$_7$CX$_8$GX$_9$X$_{10}$X$_{11}$X$_{12}$X$_{13}$X$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIV X$_{17}$X$_{18}$GCX$_{19}$X$_{20}$X$_{21}$DX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 1),

wherein X$_1$ is F or Y; X$_2$ is F or Y; X$_3$ is E or A; X$_4$ is K or L; X$_5$ is D or E; X$_6$ is R or A; X$_7$ is P or R; X$_8$ is Y or E; X$_9$ is D or E; X$_{10}$ is K or Q; X$_{11}$ is D or A; X$_{12}$ is K or A; X$_{13}$ is R or A; X$_{14}$ is R or L; X$_{15}$ is F or Y; X$_{16}$ is K, R, or A; X$_{17}$ is K, A, Y, F, or I; X$_{18}$ is Q or K; X$_{19}$ is W or A; X$_{20}$ is L or A; X$_{21}$ is D, K, R, A, F, G, M, N, or I; X$_{22}$ is I, F, or A; X$_{23}$ is K or T; X$_{24}$ is K or E; X$_{25}$ is D or E; X$_{26}$ is S or N; and X$_{27}$ is E or Q, and wherein the variant has at least one amino acid substitution relative to a wild-type extracellular ActRIIa having the sequence of SEQ ID NO: 73 or an extracellular ActRIIa having any one of the sequences of SEQ ID NOs: 76-96.

2. The polypeptide of claim 1, wherein the variant has a sequence of GAILGRSETQECLFX$_2$NANWX$_3$X$_4$X$_5$X$_6$TNQTGVEX$_7$CX$_8$GX$_9$KX$_{11}$X$_{12}$X$_{13}$X$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVX$_{17}$ X$_{18}$GCX$_{19}$X$_{20}$X$_{21}$DX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 2),

3. The polypeptide of claim 1 or 2, wherein the variant has a sequence of GAILGRSETQECLFX$_2$NANWEX$_4$X$_5$RTNQTGVEX$_7$CX$_8$GX$_9$KDKRX$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVKX$_{18}$GCWL DDX$_{22}$NCYDRTDCVEX$_{23}$X$_{24}$X$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 3).

4. The polypeptide of any one of claims 1-3, wherein the variant has a sequence of GAILGRSETQECLFX$_2$NANWEX$_4$DRTNQTGVEX$_7$CX$_8$GX$_9$KDKRX$_{14}$HCX$_{15}$ATWX$_{16}$NISGSIEIVKX$_{18}$GCWL DDX$_{22}$NCYDRTDCVEX$_{23}$KX$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 4).

5. The polypeptide of any one of claims 1-4, wherein the variant has a sequence of GAILGRSETQECLFX$_2$NANWEX$_4$DRTNQTGVEPCX$_8$GX$_9$KDKRX$_{14}$HCFATWKNISGSIEIVKX$_{18}$GCWLDDI NCYDRTDCVEX$_{23}$KX$_{25}$X$_{26}$PX$_{27}$VYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 5).

6. The polypeptide of claim 1, wherein X$_1$ is F.

7. The polypeptide of claim 1, wherein X$_1$ is Y.

8. The polypeptide of claim 1, 6, or 7 wherein X$_{10}$ is K.

9. The polypeptide of claim 1, 6, or 7 wherein X$_{10}$ is Q.

10. The polypeptide of any one of claims 1-9, wherein X_2 is F.

11. The polypeptide of any one of claims 1-9, wherein X_2 is or Y.

12. The polypeptide of any one of claims 1, 2, and 6-11, wherein X_3 is E.

13. The polypeptide of any one of claims 1, 2, and 6-11, wherein X_3 is A.

14. The polypeptide of any one of claims 1-13, wherein X_4 is K.

15. The polypeptide of any one of claims 1-13, wherein X_4 is L.

16. The polypeptide of any one of claims 1, 2, 3, and 6-15, wherein X_5 is D.

17. The polypeptide of any one of claims 1, 2, 3, and 6-15, wherein X_5 is E.

18. The polypeptide of any one of claims 1, 2 and 6-17, wherein X_6 is R.

19. The polypeptide of any one of claims 1, 2 and 6-17, wherein X_6 is A.

20. The polypeptide of any one of claims 1-4 and 6-19, wherein X_7 is P.

21. The polypeptide of any one of claims 1-4 and 6-19, wherein X_7 is R.

22. The polypeptide of any one of claims 1-21, wherein X_8 is Y.

23. The polypeptide of any one of claims 1-21, wherein X_8 is E.

24. The polypeptide of any one of claims 1-23, wherein X_9 is D.

25. The polypeptide of any one of claims 1-23, wherein X_9 is E.

26. The polypeptide of any one of claims 1, 2 and 6-25, wherein X_{11} is D.

27. The polypeptide of any one of claims 1, 2 and 6-25, wherein X_{11} is A.

28. The polypeptide of any one of claims 1, 2 and 6-27, wherein X_{12} is K.

29. The polypeptide of any one of claims 1, 2 and 6-27, wherein X_{12} is A.

30. The polypeptide of any one of claims 1, 2 and 6-29, wherein X_{13} is R.

31. The polypeptide of any one of claims 1, 2 and 6-29, wherein X_{13} is A.

32. The polypeptide of any one of claims 1-31, wherein X_{14} is R.

33. The polypeptide of any one of claims 1-31, wherein X_{14} is L.

34. The polypeptide of any one of claims 1-4 and 6-33, wherein X_{15} is F.

35. The polypeptide of any one of claims 1-4 and 6-33, wherein X_{15} is Y.

36. The polypeptide of any one of claims 1-4 and 6-35, wherein X_{16} is K.

37. The polypeptide of any one of claims 1-4 and 6-35, wherein X_{16} is R.

38. The polypeptide of any one of claims 1-4 and 6-35, wherein X_{16} is A.

39. The polypeptide of any one of claims 1, 2 and 6-38, wherein X_{17} is K.

40. The polypeptide of any one of claims 1, 2 and 6-38, wherein X_{17} is A.

41. The polypeptide of any one of claims 1, 2 and 6-38, wherein X_{17} is Y.

42. The polypeptide of any one of claims 1, 2 and 6-38, wherein X_{17} is F.

43. The polypeptide of any one of claims 1, 2 and 6-38, wherein X_{17} is I.

44. The polypeptide of any one of claims 1-43, wherein X_{18} is Q.

45. The polypeptide of any one of claims 1-43, wherein X_{18} is K.

46. The polypeptide of any one of claims 1, 2 and 6-45, wherein X_{19} is W.

47. The polypeptide of any one of claims 1, 2 and 6-45, wherein X_{19} is A.

48. The polypeptide of any one of claims 1, 2 and 6-47, wherein X_{20} is L.

49. The polypeptide of any one of claims 1, 2 and 6-47, wherein X_{20} is A.

50. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is D.

51. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is K.

52. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is R.

53. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is A.

54. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is F.

55. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is G.

56. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is M.

57. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is N.

58. The polypeptide of any one of claims 1, 2 and 6-49, wherein X_{21} is I.

59. The polypeptide of any one of claims 1-4 and 6-58, wherein X_{22} is I.

60. The polypeptide of any one of claims 1-4 and 6-58, wherein X_{22} is F.

61. The polypeptide of any one of claims 1-4 and 6-58, wherein X_{22} is A.

62. The polypeptide of any one of claims 1-61, wherein X_{23} is K.

63. The polypeptide of any one of claims 1-61, wherein X_{23} is T.

64. The polypeptide of any one of claims 1, 2, 3, and 6-63, wherein X_{24} is K.

65. The polypeptide of any one of claims 1, 2, 3, and 6-63, wherein X_{24} is E.

66. The polypeptide of any one of claims 1-65, wherein X_{25} is D.

67. The polypeptide of any one of claims 1-65, wherein X_{25} is E.

68. The polypeptide of any one of claims 1-67, wherein X_{26} is S.

69. The polypeptide of any one of claims 1-67, wherein X_{26} is N.

70. The polypeptide of any one of claims 1-69, wherein X_{27} is E.

71. The polypeptide of any one of claims 1-69, wherein X_{27} is Q.

72. The polypeptide of any one of claims 1-71, wherein X_{23} is T, X_{24} is E, X_{25} is E, and X_{26} is N.

73. The polypeptide of any one of claims 1-71, wherein X_{23} is T, X_{24} is K, X_{25} is E, and X_{26} is N.

74. The polypeptide of any one of claims 1-73, wherein X_{17} is K.

75. The polypeptide of claim 1, wherein the variant has the sequence of any one of SEQ ID NOs: 6-72

76. The polypeptide of any one of claims 1-75, wherein the amino acid at position X_{24} is replaced with the amino acid K.

77. The polypeptide of any one of claims 1-75, wherein the amino acid at position X_{24} is replaced with the amino acid E.

78. The polypeptide of any one of claims 1-77, further comprising a C-terminal extension of one or more amino acids.

79. The polypeptide of claim 78, wherein the C-terminal extension is NP.

80. The polypeptide of claim 78, wherein the C-terminal extension is NPVTPK.

81. The polypeptide of any one of claims 1-80, further comprising an Fc domain monomer fused to the C-terminus of the polypeptide by way of a linker.

82. The polypeptide of claim 81, wherein the Fc domain monomer comprises the sequence of SEQ ID NO: 97.

83. The polypeptide of any one of claims 1-80, further comprising a wild-type Fc domain fused to the C-terminus of the polypeptide by way of a linker.

84. The polypeptide of claim 83, wherein the wild-type Fc domain comprises the sequence of SEQ ID NO: 151.

85. The polypeptide of any one of claims 1-80, further comprising an Fc domain with amino acid substitutions fused to the C-terminus of the polypeptide by way of a linker.

86. The polypeptide of claim 85, wherein the Fc domain does not form a dimer.

87. The polypeptide of any one of claims 1-80, further comprising an albumin-binding peptide fused to the C-terminus of the polypeptide by way of a linker.

88. The polypeptide of claim 87, wherein the albumin-binding peptide comprises the sequence of SEQ ID NO: 152.

89. The polypeptide of any one of claims 1-80, further comprising a fibronectin domain fused to the C-terminus of the polypeptide by way of a linker.

90. The polypeptide of claim 89, wherein the fibronectin domain comprises the sequence of SEQ ID NO: 153.

91. The polypeptide of any one of claims 1-80, further comprising a human serum albumin fused to the C-terminus of the polypeptide by way of a linker.

92. The polypeptide of claim 91, wherein the human serum albumin comprises the sequence of SEQ ID NO: 154.

93. The polypeptide of claim 81 or 82, wherein the polypeptide forms a dimer.

94. The polypeptide of any one of claims 81-93, wherein the linker is an amino acid spacer.

95. The polypeptide of claim 94, wherein the amino acid spacer is GGG, GGGA (SEQ ID NO: 98), GGGG (SEQ ID NO: 100), GGGAG (SEQ ID NO: 130), GGGAGG (SEQ ID NO: 131), or GGGAGGG (SEQ ID NO: 132).

96. The polypeptide of any one of claims 1-95, wherein the polypeptide has a serum half-life of at least 7 days.

97. The polypeptide of any one of claims 1-96, wherein the polypeptide binds to human bone morphogenetic protein 9 (BMP9) with a K_D of 200 pM or higher.

98. The polypeptide of claim 97, wherein the polypeptide binds to activin and/or myostatin and has reduced or weak binding to human BMP9.

99. The polypeptide of claim 97 or 98, wherein the polypeptide does not substantially bind to human BMP9.

100. The polypeptide of any one of claims 1-99, wherein the polypeptide binds to human activin A with a K_D of 800 pM or less.

101. The polypeptide of any one of claims 1-100, wherein the polypeptide binds to human activin B with a K_D of 800 pM or less.

102. The polypeptide of any one of claims 1-101, wherein the polypeptide binds to human GDF-11 with a K_D of 5 pM or higher.

103. A nucleic acid molecule encoding a polypeptide of any of claims 1-102.

104. A vector comprising the nucleic acid molecule of claim 103.

105. A host cell that expresses a polypeptide of any one of claims 1-102, wherein the host cell comprises a nucleic acid molecule of claim 103 or a vector of claim 104, wherein the nucleic acid molecule or vector is expressed in the host cell.

106. A method of preparing a polypeptide of any one of claims 1-102, wherein the method comprising:

a) providing a host cell comprising a nucleic acid molecule of claim 103 or a vector of claim 104, and

b) expressing the nucleic acid molecule or vector in the host cell under conditions that allow for the formation of the polypeptide.

107. A pharmaceutical composition comprising a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, or a vector of claim 104, and one or more pharmaceutically acceptable carriers or excipients.

108. The pharmaceutical composition of claim 107, wherein the polypeptide is in a therapeutically effective amount.

109. A method of increasing bone mineral density in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

110. A method of reducing bone resorption in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

111. A method of increasing bone formation in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

112. The method of any one of claims 109-111, wherein the subject has primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss.

113. A method of affecting myostatin, activin, and/or BMP9 signaling in a subject having a disease or condition involving bone damage, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

114. The method of claims 113, wherein the disease or condition is primary osteoporosis, secondary osteoporosis, osteopenia, osteopetrosis, facture, bone cancer or cancer metastasis-related bone loss, Paget's disease, renal osteodystrophy, treatment-related bone loss, diet-related bone loss, bone loss associated with the treatment of obesity, low gravity-related bone loss, or immobility-related bone loss.

115. A method of treating a subject having primary osteoporosis, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

116. A method of treating a subject having secondary osteoporosis, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

117. A method of treating a subject having osteopenia, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

118. A method of treating a subject having fracture, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

119. A method of treating a subject having bone cancer or cancer metastasis-related bone loss, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

120. A method of treating a subject having Paget's disease, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

121. A method of treating a subject having renal osteodystrophy, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

122. A method of treating a subject having treatment-related bone loss, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

123. A method of treating a subject having diet-related bone loss, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

124. A method of treating a subject having low gravity-related bone loss, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

125. A method of treating a subject having immobility-related bone loss, comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1-102, a nucleic acid molecule of claim 103, a vector of claim 104, or a pharmaceutical composition of claim 107 or 108.

126. The method of any one of claims 112, 114, and 115, wherein the primary osteoporosis is age-related osteoporosis.

127. The method of any one of claims 112, 114, and 115, wherein the primary osteoporosis is hormone-related osteoporosis.

128. The method of any one of claims 112, 114, and 116, wherein the secondary osteoporosis is immobilization-induced osteoporosis.

129. The method of any one of claims 112, 114, and 116, wherein the secondary osteoporosis is glucocorticoid-induced osteoporosis.

130. The method of any one of claims 112, 114, and 119, wherein the cancer is multiple myeloma.

131. The method of any one of claims 112, 114, and 122, wherein the treatment is FGF-21 treatment.

132. The method of any one of claims 112, 114, and 122, wherein the treatment is GLP-1 treatment.

133. The method of any one of claims 112, 114, and 122, wherein the treatment is cancer therapy.

134. The method of any one of claims 112, 114, and 122, wherein the treatment is treatment for obesity or Type-2 diabetes.

135. The method of any one of claims 112, 114, and 123, wherein the diet-related bone loss is rickets.

136. The method of any one of claims 109-135, wherein the method increases bone formation in the subject.

137. The method of any one of claims 109-136, wherein the method decreases bone resorption in the subject.

138. The method of any one of claims 109-137, wherein the method increases osteoblast activity or osteoblastogenesis.

139. The method of any one of claims 109-138, wherein the method decreases osteoclast activity or decreases osteoclastogenesis.

140. The method of any one of claims 109-139, wherein the method reduces or inhibits the binding of activin and/or myostatin to their receptors.

141. The method of any one of claims 109-140, wherein the method does not cause a vascular complication in the subject.

142. The method of claim 141, wherein the method does not increase vascular permeability or leakage.

143. The method of any one of claims 109-142, wherein the method increases bone mineral density in the subject.

144. The method of any one of claims 109-143, wherein the bone is cortical bone.

145. The method of any one of claims 109-144, wherein the bone is trabecular bone.

146. The method of any one of claims 109-145, wherein the polypeptide, nucleic acid, vector, or pharmaceutical composition is administered in an amount sufficient to increase bone density, reduce bone resorption, reduce the rate of bone resorption, increase bone formation, increase the rate of bone formation, reduce osteoclast activity, increase osteoblast activity, or affect myostatin, activin, and/or BMP9 signaling in the subject.

FIG. 1

Construct		Sequence
ActRIIA		C LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC
ActRIIB		C IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC
ActRIIA/B	GA	ILGRSETQEC LFYNANWELE RTNQTGVERC EGEKDKRLHC YATWRNISGS IEIVKKGCWL DDFNCYDRTD CVETEENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTS
ActRIIB/A		GRGEAETREC IYYNANWEKD RTNQSGLEPC YGDQDKRRHC FASWKNSSGT IELVKQGCWL DDINCYDRQE CVAKKDSPEV YFCCCEGNFC NERFTHLPEA GGPEVTYEPP PTAPT
ActRIIA/Bdelta9	GA	ILGRSETQEC LFYNANWELD RTNQTGVERC EGEKDKRLHC YATWRNISGS IEIVKKGCWL DDFNCYDRTD CVETKENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTS
ActRIIA/Bdelta9min	GA	ILGRSETQEC LFYNANWELD RTNQTGVEPC EGEKDKRLHC FATWKNISGS IEIVKKGCWL DDINCYDRTD CVETKENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTS
ActRIIA/B+	GA	ILGRSETQEC LFYNANWELE RTNQTGVERC EGEKDKRLHC YATWRNISGS IEIVAKGCWL DDFNCYDRTD CVETEENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTS
ActRIIa/b-delta-9m2	GA	ILGRSETQEC LFYNANWELD RTNQTGVERC EGEKDKRLHC YATWRNISGS IEIVKKGCWL DDINCYDRTD CVETKENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTS
ActRIIa/b-delta-9m3	GA	ILGRSETQEC LFYNANWELD RTNQTGVERC EGEKDKRLHC YATWRNISGS IEIVKKGCWL DDFNCYDRTD CVETKENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTS
ActRIIa/b-delta-9m4	GA	ILGRSETQEC LFYNANWELD RTNQTGVEPC EGEKDKRLHC YATWRNISGS IEIVKKGCWL DDFNCYDRTD CVETKENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTS
ActRIIa/bmax1	GA	ILGRSETQEC LYYNANWELE RTNQTGVERC EGEQDKRLHC YATWRNISGS IEIVKKGCWL DDFNCYDRTD CVETEENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTS
ActRIIa/bmax2	GA	ILGRSETQEC LFYNANWELE RTNQTGVERC EGEKDKRLHC YATWRNISGS IEIVKKGCWL DDFNCYDRTD CVETEENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTSGG
ActRIIa/bmax3	GA	ILGRSETQEC LFYNANWELE RTNQTGVERC EGEKDKRLHC YATWRNISGS IEIVKKGCWL DDFNCYDRTD CVETEENPQV YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPK

Blue = 11A residue into 11B

Red = 11B into 11A

Green = non 11a or 11b residue

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/60970

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/00, A61K 38/17, A61K 38/18 (2018.01)
CPC - C07K 14/705, C07K 14/71, A61K 38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 2010/0267133 A1 (KNOPF et al.) 21 October 2010 (21.10.2010) para [0005]; [0029]; SEQ ID NO: 12.	1-3, 6, 8 --- 75
A	US 2012/0121576 A1 (SEEHRA et al.) 17 May 2012 (17.05.2012) para [0010]-[0011]; Table 4; SEQ ID NO: 3.	75

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 March 2018	27 MAR 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313 1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/60970

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4, 5, 10-74, 76-146
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-3, 6-9 and 75, directed to an extracellular activin receptor type IIa (ActRIIa) variant polypeptide. The ActRIIa variant will be searched to the extent that the amino acid sequence encompasses SEQ ID NO: 6 (the first claimed sequence conforming to SEQ ID NO: 1 of claim 1). It is believed that claims 1-3, 6, 8(in part) and 75 encompass this first named invention (because for SEQ ID NO: 6, X1 is F and X10 is K), and thus these claims will be searched without fee to the extent that the ActRIIa variant encompasses SEQ ID NO: 6. Additional ActRIIa variants will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected ActRIIa variants. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be ActRIIa variant SEQ ID NO: 7 (Note, SEQ ID NO: 7 is identical to SEQ ID NO: 6 except for X5 is E instead of D (claims 1-3, 6, 8(in part) and 75).
--continued on extra sheet--

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 6, 8 and 75 limited to SEQ ID NO: 6

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

--continued from Box III: Observations where unity of invention is lacking--

The inventions listed as Group I+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

No technical features are shared between the ActRIIa amino acid sequences of Group I+ and, accordingly, this group lacks unity a priori.

Additionally, even if Group I+ was considered to share the technical features of including: a polypeptide comprising an extracellular ActRIIa variant having a sequence of SEQ ID NO: 1, wherein the variant has at least one amino acid substitution relative to a wild-type extracellular ActRIIa having the sequence of SEQ ID NO: 73 or an extracellular ActRIIa having any one of the sequences of SEQ ID *NOs: 76-96, these shared technical features are previously disclosed by US 2010/0267133 A1 to Knopf et al., (hereinafter Knopf).*

Knopf teaches a polypeptide comprising an extracellular activin receptor type IIa (ActRIIa) variant (para [0005] "a soluble ActRII (e.g., ActRIIA or ActRIIB) polypeptide...A soluble ActRII polypeptide may include a functional fragment of a natural ActRII polypeptide, such as one comprising at least 10, 20 or 30 amino acids of a sequence selected from SEQ ID NOs: 1-4 and 9-12 or a sequence of SEQ ID NOs: 1 or 2, lacking the C-terminal 10 to 15 amino acids (the "tail"). A soluble ActRII polypeptide may include one or more alterations in the amino acid sequence (e.g., in the ligand-binding domain) relative to a naturally occurring ActRII polypeptide...In a preferred embodiment, an ActRII-Fc fusion comprises...the extracellular ActRII domain"), the variant having a sequence of GAILGRSETQECLX1X2NANWX3X4XsX5TNQTGVEX7CX8GX9X10X11X12X13X14HCX15ATWX16NISGSIEIVX17X18GCX19X20X21 DX22NCYDRTDCVE X23X24X2sX25PX2NYFCCCEGNMCNEKFSYFPEMEVTQPTS (SEQ ID NO: 1), wherein X1 is F or Y; X2 is F or Y; X3 is E or A; X4 is K or L; Xs is D or E; X6 is R or A; X7 is P or R; X8 is Y or E; X9 is D or E; X10 is K or Q; X11 is D or A; X12 is K or A; X13 is R or A; X14 is R or L; X15 is For Y; X16 is K, R, or A; X17 is K, A, Y, F, or I; X18 is Q or K; X19 is W or A; X20 is L or A; X21 is D, K, R, A, F, G, M, N, or I; X22 is I, F, or A; X23 is K or T; X24 is K or E; X25 is D or E; X26 is S or N; and X27 is E or Q, and wherein the variant has at least one amino acid substitution relative to a wild-type extracellular ActRIIa having the sequence of SEQ ID NO: 73 or an extracellular ActRIIa having any one of the sequences of SEQ ID NOs: 76-96 (para [0005] " a polypeptide having an amino acid sequence selected from SEQ ID NOs: 1-2 and 9-12...A soluble ActRII polypeptide may include one or more alterations in the amino acid sequence (e.g., in the ligand-binding domain) relative to a *naturally occurring ActRII polypeptide*"; SEQ ID NO: 12 has 100% identity to claimed SEQ ID NO: 1 *wherein each variable residue is the* first alternate residue, and 100% identity to SEQ ID NO: 73).

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ and II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, claims 4, 5, 10-74, 76-146 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).