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(71) Applicants: **CELGENE CORPORATION** [US/US]; 86 Morris Avenue, Summit, NJ 07901 (US). **WASHINGTON UNIVERSITY** [US/US]; One Brookings Drive, St. Louis, MO 63130 (US).

(72) Inventors: **HRUSKA, Keith**; 12437 Conway Road, Creve Coeur, MO 63141 (US). **FANG, Yifu**; 4394 West Pine #102, St. Louis, MO 63108 (US). **SMITH, William**; 1521 Glasco Turnpike, Woodstock, NY 12498 (US). **CHEN, Nianhang**; 34 Smithfield Court, Basking Ridge, NJ 07920 (US).

(74) Agents: **MARTINEK, Sebastian** et al.; Jones Day, 222 East 41st Street, New York, NY 10017-6702 (US).

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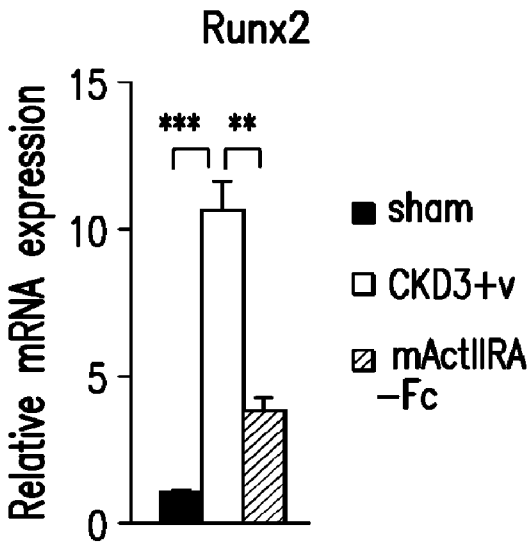


FIG. 4A

(57) Abstract: Provided herein are methods of treating diseases associated with vascular calcification and/or cardiovascular disease, and/or bone resorption in a subject, by using the level of a biomarker, in particular, snail homolog I (Snail), phosphosmad2, phosphosmad3, urinary protein, dickkopf homolog I (Dkkl), collagen type 1 alphal (Collal), activin (i.e.free activin), runt-related transcription factor 2 (Runx2), alkaline phosphatase (Alp), bone-specific alkaline phosphatase (BSAP), C-terminal type 1 collagen telopeptide (CTX), osterix, Klotho, alpha-smooth muscle actin (alpha-SMA), myocardin (JVIYOCD), activin receptor type 2A (ActRIIA), axis inhibition protein 2 (Axin2), and/or smooth muscle protein 22-alpha (Sm22-alpha), as an indicator(s) of responsiveness of the subject to the treatment, efficacy of the treatment, or appropriate dosage tor the treatment with an activin type II receptor signaling inhibitor.

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TREATMENT OF CARDIOVASCULAR DISEASE USING ACTRII LIGAND TRAPS

1. CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of United States Provisional Patent Application No. 62/062,021, filed October 9, 2014; United States Provisional Patent Application No. 62/078,321, filed November 11, 2014; United States Provisional Patent Application No. 62/103,515, filed January 14, 2015; United States Provisional Patent Application No. 62/167,052, filed May 27, 2015; and United States Provisional Patent Application No. 62/170,015, filed June 2, 2015, the entire contents of each of which are incorporated herein by reference and for all purposes.

2. GOVERNMENT LICENSE RIGHTS

[0002] This invention was made with government support under grant numbers DK070790 and DK089137 awarded by National Institutes of Health. The government has certain rights in the invention.

3. SEQUENCE LISTING

[0003] The present application is being filed with a Sequence Listing submitted as file name 12827_934_228_SeqListing.txt, of size 208 kilobytes, which was created on October 6, 2015. The Sequence Listing is incorporated herein by reference in its entirety and for all purposes.

4. FIELD

[0004] Provided herein are methods of treating and/or preventing cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, and/or cardiovascular disease associated with and/or resulting from renal disease in a subject, comprising administering to the subject an activin type II receptor signaling inhibitor (ActRII signaling inhibitor, *e.g.*, an activin ligand trap). More specifically, provided herein are methods for selecting subjects for the treatment of cardiovascular disease, vascular calcification, elevated levels of arterial stiffness, left ventricular hypertrophy, cardiovascular disease associated with and/or resulting from vascular calcification, cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness, cardiovascular disease associated with and/or resulting from left ventricular hypertrophy, and/or cardiovascular disease associated with and/or resulting from renal disease, by using the level and/or activity of one or more biomarkers, in particular, snail homolog 1 (Snai1), phosphosmad2, phosphosmad3, urinary

protein, dickkopf homolog 1 (Dkk1), collagen type 1 alpha 1 (Col1a1), activin (*e.g.*, free activin), runt-related transcription factor 2 (Runx2), alkaline phosphatase (Alp), bone-specific alkaline phosphatase (BSAP), Osterix, C-terminal type 1 collagen telopeptide (CTX), Klotho, alpha-smooth muscle action (alpha-SMA), myocardin (MYOCD), axis inhibition protein 2 (Axin2), and/or smooth muscle protein 22-alpha (Sm22-alpha), as an indicator(s) of responsiveness of a subject to the treatment, efficacy of the treatment, or appropriate dosage for the treatment with an activin type II receptor (ActRII) signaling inhibitor. Provided herein are methods of reducing bone resorption in a subject, comprising administering to the subject an ActRII signaling inhibitor. More specifically, provided herein are methods for selecting subjects for the reduction of bone resorption, by using the level and/or activity of one or more biomarkers, in particular, Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, Osterix, CTX, Klotho, alpha-SMA, MYOCD, and/or Sm22-alpha, as an indicator(s) of responsiveness of a subject to the treatment, efficacy of the treatment, or appropriate dosage for the treatment with an ActRII signaling inhibitor.

5. BACKGROUND

[0005] A common complication of renal disease involving anemia is vascular calcification, which leads, in many cases, to cardiovascular disease. In renal subjects, atherosclerosis and the resulting cardiovascular disease can result in significant mortality apart from mortality associated with renal disease *per se*. As such, there is a continued need for the discovery and development of new drugs and treatment and/or prevention methods for cardiovascular disease in renal subjects. Additionally, because renal subjects frequently suffer from anemia, it would be beneficial to treat the anemia and the attendant cardiovascular disease with a single therapeutic, a capability not possessed by currently-used erythropoiesis-stimulating agents (ESAs) such as erythropoietin.

[0006] Two related type II receptors, ActRIIA and ActRIIB, have been identified as the type II receptors for activins (Mathews and Vale, 1991, Cell 65:973-982; Attisano *et al.*, 1992, Cell 68: 97-108). Besides activins, ActRIIA and ActRIIB can biochemically interact with several other TGF-beta family proteins, including BMP7, Nodal, GDF8, and GDF11 (Yamashita *et al.*, 1995, J. Cell Biol. 130:217-226; Lee and McPherron, 2001, Proc. Natl. Acad. Sci. 98:9306-9311; Yeo and Whitman, 2001, Mol. Cell 7: 949-957; Oh *et al.*, 2002, Genes Dev. 16:2749-54). ALK4 is the primary type I receptor for activins, particularly for activin A, and ALK-7 may serve as a receptor for activins as well, particularly for activin B.

[0007] An activin ligand trap, consisting of a humanized fusion-protein consisting of the extracellular domain of activin-receptor type IIA (ActRIIA) and the human IgG1 Fc (referred to herein as ActRIIA-hFc or "Sotatercept"; SEQ ID NO: 7), is currently being evaluated in phase II clinical trials for treatment of subjects with anemia and bone disorders associated with end-stage renal disease (ESRD) as well as for those subjects with beta-thalassemia. In healthy post-menopausal women, ActRIIA-hFc was shown to significantly increase hematocrit (Hct) and hemoglobin (Hgb) as well as bone mineral density.

6. SUMMARY

[0008] Provided herein are methods for treating and/or preventing vascular calcification in a subject, comprising administering to the subject a pharmaceutically effective dose of an activin receptor type II (ActRII) signaling inhibitor (*e.g.*, an activin ligand trap), wherein the subject has: (a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population; (b) elevated levels of Alp as compared to levels of Alp in a reference population; (c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population; (d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population; (e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population; (f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population; (g) elevated levels of activin (*e.g.*, free activin) as compared to levels of activin in a reference population; (h) elevated levels of BSAP as compared to levels of BSAP in a reference population; (i) elevated levels of Osterix as compared to levels of Osterix in a reference population; (j) elevated levels of CTX as compared to levels of CTX in a reference population; (k) decreased levels of Klotho as compared to levels of Klotho in a reference population; (l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population; (m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; (n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population; (o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population; (p)elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or (q) decreased levels of activin receptor type 2A (ActRIIA) as compared to levels of ActRIIA in a reference population. *See*, *e.g.*, Section 8.6 for a description of the reference population.

[0009] Provided herein are methods for treating and/or preventing cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an activin receptor type II (ActRII) signaling inhibitor (*e.g.*, an activin ligand trap), wherein the

subject has: (a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population; (b) elevated levels of Alp as compared to levels of Alp in a reference population; (c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population; (d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population; (e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population; (f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population; (g) elevated levels of activin (*e.g.*, free activin) as compared to levels of activin in a reference population; (h) elevated levels of BSAP as compared to levels of BSAP in a reference population; (i) elevated levels of Osterix as compared to levels of Osterix in a reference population; (j) elevated levels of CTX as compared to levels of CTX in a reference population; (k) decreased levels of Klotho as compared to levels of Klotho in a reference population; (l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population; (m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; (n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population; (o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population; (p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or (q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

[0010] Provided herein are methods for treating and/or preventing cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an activin receptor type II (ActRII) signaling inhibitor (*e.g.*, an activin ligand trap), wherein the subject has: (a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population; (b) elevated levels of Alp as compared to levels of Alp in a reference population; (c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population; (d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population; (e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population; (f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population; (g) elevated levels of activin (*e.g.*, free activin) as compared to levels of activin in a reference population; (h) elevated levels of BSAP as compared to levels of BSAP in a reference population; (i) elevated levels of Osterix as compared to levels of Osterix in a reference population; (j) elevated levels of CTX as compared to levels of CTX in a reference population; (k) decreased levels of Klotho as compared to levels of Klotho in a reference population; (l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population; (m) decreased levels of MYOCD as compared to

levels of MYOCD in a reference population; (n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population; (o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population; (p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or (q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population, and wherein the cardiovascular disease is associated with and/or results from vascular calcification.

[0011] Provided herein are methods for treating and/or preventing cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an activin receptor type II (ActRII) signaling inhibitor (*e.g.*, an activin ligand trap), wherein the subject has: (a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population; (b) elevated levels of Alp as compared to levels of Alp in a reference population; (c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population; (d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population; (e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population; (f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population; (g) elevated levels of activin (*e.g.*, free activin) as compared to levels of activin in a reference population; (h) elevated levels of BSAP as compared to levels of BSAP in a reference population; (i) elevated levels of Osterix as compared to levels of Osterix in a reference population; (j) elevated levels of CTX as compared to levels of CTX in a reference population; (k) decreased levels of Klotho as compared to levels of Klotho in a reference population; (l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population; (m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; (n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population; (o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population; (p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or (q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population, and wherein the cardiovascular disease is associated with and/or results from renal disease.

[0012] Provided herein are methods for reducing bone resorption in a subject, comprising administering to the subject a pharmaceutically effective dose of an activin receptor type II (ActRII) signaling inhibitor (*e.g.*, an activin ligand trap), wherein the subject has: (a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population; (b) elevated levels of Alp as compared to levels of Alp in a reference population; (c) elevated levels of Snai1 as

compared to levels of Snai1 in a reference population; (d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population; (e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population; (f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population; (g) elevated levels of activin (*e.g.*, free activin) as compared to levels of activin in a reference population; (h) elevated levels of BSAP as compared to levels of BSAP in a reference population; (i) elevated levels of Osterix as compared to levels of Osterix in a reference population; (j) elevated levels of CTX as compared to levels of CTX in a reference population; (k) decreased levels of Klotho as compared to levels of Klotho in a reference population; (l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population; (m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; (n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population; (o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population; (p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or (q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

[0013] Provided herein are methods for treating and/or preventing arterial stiffness in a subject, comprising administering to the subject a pharmaceutically effective dose of an activin receptor type II (ActRII) signaling inhibitor (*e.g.*, an activin ligand trap), wherein the subject has: (a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population; (b) elevated levels of Alp as compared to levels of Alp in a reference population; (c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population; (d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population; (e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population; (f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population; (g) elevated levels of activin (*e.g.*, free activin) as compared to levels of activin in a reference population; (h) elevated levels of BSAP as compared to levels of BSAP in a reference population; (i) elevated levels of Osterix as compared to levels of Osterix in a reference population; (j) elevated levels of CTX as compared to levels of CTX in a reference population; (k) decreased levels of Klotho as compared to levels of Klotho in a reference population; (l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population; (m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; (n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population; (o) elevated levels of phosphosmad3 as

compared to levels of phosphosmad3 in a reference population; (p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or (q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

[0014] Provided herein are methods for treating and/or preventing left ventricular hypertrophy in a subject, comprising administering to the subject a pharmaceutically effective dose of an activin receptor type II (ActRII) signaling inhibitor (*e.g.*, an activin ligand trap), wherein the subject has: (a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population; (b) elevated levels of Alp as compared to levels of Alp in a reference population; (c) elevated levels of Snail as compared to levels of Snail in a reference population; (d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population; (e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population; (f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population; (g) elevated levels of activin (*e.g.*, free activin) as compared to levels of activin in a reference population; (h) elevated levels of BSAP as compared to levels of BSAP in a reference population; (i) elevated levels of Osterix as compared to levels of Osterix in a reference population; (j) elevated levels of CTX as compared to levels of CTX in a reference population; (k) decreased levels of Klotho as compared to levels of Klotho in a reference population; (l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population; (m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; (n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population; (o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population; (p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or (q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

[0015] In certain embodiments, the pharmaceutically effective dose of the ActRII signaling inhibitor is about 15 mg, about 30 mg, about 45 mg, about 60 mg, about 75 mg, about 90 mg, or about 1 g, or about 0.1 mg/kg, about 0.13 mg/kg, about 0.2 mg/kg, about 0.26 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg. In certain embodiments, the pharmaceutically effective dose of the ActRII signaling inhibitor is about 0.1 mg/kg. In certain embodiments, the pharmaceutically effective dose of the ActRII signaling inhibitor is about 0.3 mg/kg. In certain

embodiments, the pharmaceutically effective dose of the ActRII signaling inhibitor is about 0.5 mg/kg. In certain embodiments, the pharmaceutically effective dose of the ActRII signaling inhibitor is about 0.7 mg/kg.

[0016] In certain embodiments, the pharmaceutically effective dose is administered via injection. In certain embodiments, the pharmaceutically effective dose is administered (i) once every 28 days; or (ii) once every 42 days. In certain embodiments, the pharmaceutically effective dose is administered once every 14 days. In certain embodiments, the pharmaceutically effective dose is administered once every 21 days. In certain embodiments, the pharmaceutically effective dose is administered continuously and/or indefinitely.

[0017] In certain embodiments, the elevated levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein and/or Osterix, are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, 100%, 200%, or 500% greater than the levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix, respectively, in the reference population.

[0018] In certain embodiments, the elevated levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 200%, or 500% greater than the levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix, respectively, in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population.

[0019] In certain embodiments, the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the reference population.

[0020] In certain embodiments, the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

[0021] Provided herein are methods for treating and/or preventing vascular calcification in a subject, wherein the method comprises: (a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject; (b) taking a first measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; (c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and (d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor. In certain embodiments, the adjusted dose is based on the detected change between the first measurement and the second measurement. In certain embodiments, the dose is adjusted as described in Section 8.3.4.

[0022] Provided herein are methods for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises: (a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject; (b) taking a first measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; (c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and (d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor. In certain embodiments, the adjusted dose is based on the detected change between the first measurement and the second measurement. In certain embodiments, the dose is adjusted as described in Section 8.3.4.

[0023] Provided herein are methods for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises: (a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject; (b) taking a first measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; (c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-

alpha in the subject; and (d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor; and wherein the cardiovascular disease is associated with and/or results from vascular calcification. In certain embodiments, the adjusted dose is based on the detected change between the first measurement and the second measurement. In certain embodiments, the dose is adjusted as described in Section 8.3.4.

[0024] Provided herein are methods for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises: (a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject; (b) taking a first measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; (c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and (d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor; and wherein the cardiovascular disease is associated with and/or results from renal disease. In certain embodiments, the adjusted dose is based on the detected change between the first measurement and the second measurement. In certain embodiments, the dose is adjusted as described in Section 8.3.4.

[0025] Provided herein are methods for reducing bone resorption in a subject, wherein the method comprises: (a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject; (b) taking a first measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; (c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and (d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor. In certain embodiments, the bone resorption is assessed as described in Section 8.6. In certain embodiments, the adjusted dose is based on the detected change between the first measurement and the second measurement. In certain embodiments, the dose is adjusted as described in Section 8.3.4.

[0026] Provided herein are methods for treating and/or preventing arterial stiffness in a subject, wherein the method comprises: (a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject; (b) taking a first measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; (c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and (d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor. In certain embodiments, the arterial stiffnesss is assessed as described in Section 8.6. In certain embodiments, the adjusted dose is based on the detected change between the first measurement and the second measurement. In certain embodiments, the dose is adjusted as described in Section 8.3.4.

[0027] Provided herein are methods for treating and/or preventing left ventricular hypertrophy in a subject, wherein the method comprises: (a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject; (b) taking a first measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; (c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and (d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor. In certain embodiments, the adjusted dose is based on the detected change between the first measurement and the second measurement. In certain embodiments, the dose is adjusted as described in Section 8.3.4.

[0028] In certain embodiments, the initial dose of the ActRII signaling inhibitor is about 15 mg, about 30 mg, about 45 mg, about 60 mg, about 75 mg, about 90 mg, or about 1 g or about 0.1 mg/kg, about 0.13 mg/kg, about 0.2 mg/kg, about 0.26 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg. In certain embodiments, the initial dose of the ActRII signaling inhibitor is about 0.1 mg/kg. In certain embodiments, the initial dose of the ActRII signaling inhibitor is about 0.3

mg/kg. In certain embodiments, the initial dose of the ActRII signaling inhibitor is about 0.5 mg/kg. In certain embodiments, the initial dose of the ActRII signaling inhibitor is about 0.7 mg/kg.

[0029] In certain embodiments, the initial dose is administered via injection. In certain embodiments, the initial dose is administered (i) once every 28 days; or (ii) once every 42 days. In certain embodiments, the initial dose is administered once every 14 days. In certain embodiments, the initial dose is administered once every 21 days.

[0030] In certain embodiments, the adjusted dose of the ActRII signaling inhibitor is greater than the initial dose if: (a) the level of Runx2 is elevated as compared to the level of Runx2 in a reference population; (b) the level of Alp is elevated as compared to the level of Alp in a reference population; (c) the level of Snai1 is elevated as compared to the level of Snai1 in a reference population; (d) the level of phosphosmad2 is elevated as compared to the level of phosphosmad2 in a reference population; (e) the level of Dkk1 is elevated as compared to the level of Dkk1 in a reference population; (f) the level of Col1a1 is elevated as compared to the level of Col1a1 in a reference population; (g) the level of activin is elevated as compared to the level of activin in a reference population; (h) the level of BSAP is elevated as compared to the level of BSAP in a reference population; (i) the level of CTX is elevated as compared to the level of CTX in a reference population; (j) the level of Osterix is elevated as compared to the level of Osterix in a reference population; (k) the level of Klotho is decreased as compared to the level Klotho in a reference population; (l) the level of alpha-SMA is decreased as compared to the level of alpha-SMA in a reference population; (m) the level of MYOCD is decreased as compared to the level of MYOCD in a reference population; (n) the level of Sm22-alpha is decreased as compared to the level of Sm22-alpha in a reference population; (o) the level of phosphosmad3 is elevated as compared to the level of phosphosmad3 in a reference population; (p) the level of urinary protein is elevated as compared to the level of urinary protein in a reference population; (q) the level of ActRIIA is decreased as compared to the level ActRIIA in a reference population; and/or (r) the level of Axin2 is decreased as compared to the level Axin2 in a reference population.

[0031] In certain embodiments, the adjusted dose is about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, or about 35 mg greater than the initial dose, or about 0.05 mg/kg, about 0.1 mg/kg, about 0.15 mg/kg, about 0.25 mg/kg, about 0.3 mg/kg, about 0.35 mg/kg, about 0.4 mg/kg, or about 0.5 mg/kg greater than the initial dose. In certain embodiments, the adjusted

dose is administered more frequently than the initial dose. In certain embodiments, the adjusted dose is administered every 5, 10, 15, 20, 25, 28, 30, 35, or 40 days.

[0032] In certain embodiments, the adjusted dose of the ActRII signaling inhibitor is less than the initial dose if: (a) the level of Runx2 is decreased as compared to the level of Runx2 in a reference population; (b) the level of Alp is decreased as compared to the level of Alp in a reference population; (c) the level of BSAP is decreased as compared to the level of BSAP in a reference population; (d) the level of Snai1 is decreased as compared to the level of Snai1 in a reference population; (e) the level of phosphosmad2 is decreased as compared to the level of phosphosmad2 in a reference population; (f) the level of Dkk1 is decreased as compared to the level of Dkk1 in a reference population; (g) the level of col1a1 is decreased as compared to the level of col1a1 in a reference population; (h) the level of activin (*e.g.*, free activin) is decreased as compared to the level of activin (*e.g.*, free activin) in a reference population; (i) the level of CTX is decreased as compared to the level of CTX in a reference population; (j) the level of Osterix is decreased as compared to the level of Osterix in a reference population; (k) the level of Klotho is elevated as compared to the level of Klotho in a reference population; (l) the level of alpha-SMA is elevated as compared to the level of alpha-SMA in a reference population; (m) the level of MYOCD is elevated as compared to the level of MYOCD in a reference population; (n) the level of Sm22-alpha is elevated as compared to the level of Sm22-alpha in a reference population; (o) the level of phosphosmad3 is decreased as compared to level of phosphosmad3 in a reference population; (p) the level of urinary protein is decreased as compared to level of urinary protein in a reference population; (q) the level of ActRIIA is elevated as compared to level of ActRIIA in a reference population; and/or (r) the level of Axin2 is elevated as compared to level of Axin2 in a reference population.

[0033] In certain embodiments, the adjusted dose is about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, or about 35 mg less than the initial dose, or about 0.05 mg/kg, about 0.1 mg/kg, about 0.15 mg/kg, about 0.25 mg/kg, about 0.3 mg/kg, about 0.35 mg/kg, about 0.4 mg/kg, or about 0.5 mg/kg less than the initial dose. In certain embodiments, the adjusted dose is administered less frequently than the initial dose. In certain embodiments, the adjusted dose is administered every 30, 35, 40, 42, 50, 60, 70, 80, or 90 days. In certain embodiments, the adjusted dose is administered continuously and/or indefinitely.

[0034] In certain embodiments, the first measurement is taken prior to the commencement of the treatment. In certain embodiments, the first measurement is taken

immediately after commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof. In certain embodiments, the second measurement is taken immediately after commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months thereof.

[0035] In certain embodiments, (a) the elevated levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, 100%, 200%, or 500% greater than the levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix, respectively, in the reference population; and/or (b) the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, or 100% less than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population.

[0036] In certain embodiments, (a) the elevated levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 200%, or 500% greater than the levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix, respectively, in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population; and/or (b) the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

[0037] In certain embodiments, (a) the elevated levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, 100%, 200%, or 500% greater than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the reference population; and/or (b) the decreased levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix are about 10%, 20%, 25%, 30%,

40%, 50%, 60%, 70%, 75%, 80% 90%, or 100% less than the levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix, respectively, in a reference population.

[0038] In certain embodiments, (a) the elevated levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 200%, or 500% greater than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population; and/or (b) the decreased levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix, respectively, in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

[0039] In certain embodiments, the level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha is the protein level of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha, respectively. In certain embodiments, the protein level is determined by enzyme-linked immunosorbent assay (ELISA). In certain embodiments, the ELISA is performed with (a) Runx2-specific antibody SC-390715 (Santa Cruz) to determine Runx2 levels; (b) Alp-specific antibody SC-98652 (Santa Cruz) to determine Alp levels; (c) Snai1-specific antibody sc-393172 (Santa Cruz) to determine Snai1 levels; (d) phosphosmad2-specific antibody sc-101801 (Santa Cruz) to determine phosphosmad2 levels; (e) Dkk1-specific antibody sc-374574 (Santa Cruz) to determine Dkk1 levels; (f) Col1a1-specific antibody sc-8784 (Santa Cruz) to determine col1a1 levels; (g) activin-specific antibody A1594 (Sigma Aldrich) to determine activin levels; (h) BSAP-specific antibody SC-98652 (Santa Cruz) to determine BSAP levels; (i) CTX-specific antibody ABIN1173415 (Antibodies Online) to determine CTX levels; (j) Osterix-specific antibody SC-22538 (Santa Cruz) to determine Osterix levels; (k) Klotho-specific antibody SC-22218 (Santa Cruz) to determine Klotho levels; (l) alpha-SMA-specific antibody SC- 53142 (Santa Cruz) to determine alpha-SMA levels; (m) MYOCD-specific antibody SC-21561 (Santa

Cruz) to determine MYOCD levels; (n) Sm22-alpha-specific antibody SC-271719 (Santa Cruz) to determine Sm22-alpha levels; (o) phosphosmad3-specific antibody sc-11769 (Santa Cruz) to determine phosphosmad3 levels; and/or (p) ActRIIA-specific antibody ab 135634 (Abcam) to determine ActRIIA levels.

[0040] In certain embodiments, the level of Snai1, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha is the mRNA level of Snai1, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha, respectively. In certain embodiments, the mRNA level is determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR). In certain embodiments, the qRT-PCR is performed with (a) Runx2-specific primers (SEQ ID NOS: 48 and 49) to determine Runx2 levels; (b) Alp-specific primers (SEQ ID NOS: 50 and 51) to determine Alp levels; (c) Snai1-specific primers (SEQ ID NOS: 78 and 79) to determine Snai1 levels; (d) Dkk1-specific primers (SEQ ID NOS: 80 and 81) to determine Dkk1 levels; (e) col1a1-specific primers (SEQ ID NOS: 82 and 83) to determine col1a1 levels; (f) activin-specific primers (SEQ ID NOS: 84 and 85) to determine activin levels; (g) Osterix-specific primers (SEQ ID NOS: 52 and 53) to determine Osterix levels; (h) Klotho-specific primers (SEQ ID NOS: 54 and 55) to determine Klotho levels; and/or (i) Sm22-alpha-specific primers (SEQ ID NOS: 56 and 57) to determine Sm22-alpha levels.

[0041] In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, ActRIIA, Axin2, and/or alpha-SMA levels are in a tissue. In certain embodiments, the tissue is aorta. In certain embodiments, the tissue is kidney. In certain embodiments, the tissue is bone. In certain embodiments, the tissue is serum. In a preferred embodiment, the Runx2, Dkk1, Alp, osterix, sm22-alpha, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, levels and/or activity are in aorta. In a preferred embodiment, the phosphosmad3, urinary protein, phosphosmad2, ActRIIA, Axin2, and col1a1 levels and/or activity are in kidney. In a preferred embodiment, the activin levels and/or activity are in serum.

[0042] In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, ActRIIA, Axin2, and/or alpha-SMA levels are in aorta.

[0043] In certain embodiments, the vascular calcification is calcific atherosclerosis, calcific medial vasculopathy (also known as Mönckeberg's medial calcific sclerosis), medial calcification, elastocalcinosis, calcific uremic arterolopathy, calcific aortic valvular stenosis, or portal vein calcification.

[0044] In certain embodiments, the cardiovascular disease is a disease associated with vascular calcification such as atherosclerosis, hyperlipidemia, osteoporosis, hypertension, inflammation, type 2 diabetes mellitus, end-stage renal disease, required amputation, pseudoxanthoma elasticum, congenital bicuspid valve, rheumatic heart disease, portal hypertension, or liver disease.

[0045] In certain embodiments, the cardiovascular disease is secondary to chronic kidney disease. In certain embodiments, the chronic kidney disease is stage 3, 4, or 5 chronic kidney disease. In certain embodiments, the chronic kidney disease is chronic kidney disease mineral and bone disease.

[0046] In certain embodiments, the subject has high turnover bone disease, *e.g.*, high-turnover renal osteodystrophy (ROD).

[0047] Also provided herein is a method of treating high-turnover bone disease, *e.g.*, high-turnover ROD, comprising administering to a pharmaceutically effective amount of an ActRII signaling inhibitor to a subject.

[0048] In certain embodiments, the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of: (a) 90% identical to SEQ ID NO:2; (b) 95% identical to SEQ ID NO:2; (c) 98% identical to SEQ ID NO:2; (d) SEQ ID NO:2; (e) 90% identical to SEQ ID NO:3; (f) 95% identical to SEQ ID NO:3; (g) 98% identical to SEQ ID NO:3; (h) SEQ ID NO:3; (i) 90% identical to SEQ ID NO:6; (j) 95% identical to SEQ ID NO:6; (k) 98% identical to SEQ ID NO:6; (l) SEQ ID NO:6; (m) 90% identical to SEQ ID NO:7; (n) 95% identical to SEQ ID NO:7; (o) 98% identical to SEQ ID NO:7; (p) SEQ ID NO:7; (q) 90% identical to SEQ ID NO:12; (r) 95% identical to SEQ ID NO:12; (s) 98% identical to SEQ ID NO:12; (t) SEQ ID NO:12; (u) 90% identical to SEQ ID NO:17; (v) 95% identical to SEQ ID NO:17; (w) 98% identical to SEQ ID NO:17; (x) SEQ ID NO:17; (y) 90% identical to SEQ ID NO:20; (z) 95% identical to SEQ ID NO:20; (aa) 98% identical to SEQ ID NO:20; (bb) SEQ ID NO:20; (cc) 90% identical to SEQ ID NO:21; (dd) 95% identical to SEQ ID NO:21; (ee) 98% identical to SEQ ID NO:21; and (ff) SEQ ID NO:21. In certain embodiments, the ActRII signaling inhibitor is a polypeptide comprising the amino

acid sequence of SEQ ID NO:7. In certain embodiments, the ActRII signaling inhibitor is a humanized fusion-protein consisting of the extracellular domain of ActRIIA and the human IgG1 Fc domain.

[0049] In certain embodiments, the ActRII signaling inhibitor is Sotatercept (SEQ ID NO: 7; *see, e.g.*, Section 9). Sotatercept is an activin ligand trap that is useful in the methods described herein (*see* Section 8).

[0050] In certain embodiments, the subject is human.

7. BRIEF DESCRIPTION OF THE FIGURES

[0051] **Fig. 1A** depicts increased Activin-A levels (pg/ml) in the interstitial fibrosis model of kidney disease (CKD-3) as compared to wild-type and sham models. **Fig. 1B** depicts increased Activin-A levels (pg/ml) in the Alports model of kidney disease (Alport200d) as compared to wildtype (WT) and increased Activin-A levels upon treatment with BMP-7.

[0052] **Fig. 2** depicts the increased levels of Activin A (inhibin beta) mRNA in the kidney and aorta of a CKD-3 model (CKD-3 V) as compared to sham. Treatment with mActRIIA-Fc decreases Activin A mRNA in a CKD-3 model (mActRIIA-Fc).

[0053] **Fig. 3A** depicts the level of ACTRII in wildtype mouse aorta. **Fig. 3B** depicts the level of ACTRII in sham-treated mouse aorta. **Fig. 3C** depicts the increased level of ACTRII in mouse aorta of a CKD-3 model as compared to Fig. 3A and/or Fig. 3B. **Fig. 3D** depicts the decreased level of ACTRII in mouse aorta of a CKD-3 model treated mActRIIA-Fc as compared to Fig. 3C.

[0054] **Fig. 4A** depicts the mRNA levels of Runx2 in a mouse model of chronic kidney disease in the presence (CKD3+ActRII-Fc) and absence (CKD3+v) of treatment with an inhibitor of ActRII signaling. **Fig. 4B** depicts the mRNA levels of Alp in a mouse model of chronic kidney disease in the presence (CKD3+ActRII-Fc) and absence (CKD3+v) of treatment with an inhibitor of ActRII signaling. **Fig. 4C** depicts the mRNA levels of Klotho in a mouse model of chronic kidney disease in the presence (CKD3+ActRII-Fc) and absence (CKD3+v) of treatment with an inhibitor of ActRII signaling. **Fig. 4D** depicts the mRNA levels of myocardin (MYOCD) in a mouse model of chronic kidney disease in the presence (CKD3+ActRII-Fc) and absence (CKD3+v) of treatment with an inhibitor of ActRII signaling. **Fig. 4E** depicts the mRNA levels of Sm22-alpha (SM22α) in a mouse model of chronic kidney disease in the presence (CKD3+ActRII-Fc) and absence (CKD3+v) of treatment with an inhibitor of ActRII signaling.

[0055] **Fig. 4F** depicts the protein levels of actin alpha-smooth muscle, Runx2, Klotho, MYOCD, and alpha-tubulin in a model of CKD-3 treated with an inhibitor of ActRII signaling (CKD3+ActRII-Fc) as compared to CKD3 treated with vehicle alone (CKD3+vehicle) or sham.

[0056] **Fig. 5A** depicts the CKD-3-induced vascular calcification upon treatment with a vehicle (veh. Rx). **Fig. 5B** depicts decreased CKD-3-induced vascular calcification upon treatment with an inhibitor of ActRII signaling (mActRIIA-Fc Rx).

[0057] **Fig. 6A** depicts the CKD-3-induced increase in calcium levels (CKD-3 22 wk) as compared to wildtype (wt), sham, CKD-3 model treated with a vehicle (CKD-3 Veh). Treatment with mActRIIA-Fc decreases CKD-3-induced accumulation of calcium (mActRIIA-Fc). **Fig. 6B** depicts the bone volume of wildtype mice (wt), sham-treated mice (sham), a mouse model of CKD-3 (CKD-3), or a mouse model CKD-3 treated with an inhibitor of ActRII signaling (mActRIIA-Fc). **Fig. 6C** depicts the osteoclast number of wildtype mice (wt), sham-treated mice (sham), a mouse model of CKD-3 (CKD-3), or a mouse model of CKD-3 treated with an inhibitor of ActRII signaling (mActRIIA-Fc). **Fig. 6D** depicts the osteoclast surface of wildtype mice (wt), sham-treated mice (sham), a mouse model of CKD-3 (CKD-3), or a mouse model of CKD-3 treated with an inhibitor of ActRII signaling (mActRIIA-Fc).

[0058] **Fig. 7A** depicts the strategy for cell lineage tracing as performed in GNZ mice (Stoller et al, Genesis, 2008) bred to endothelial specific Tie2-Cre (also referred to as "Tek-Cre") mice. **Fig. 7B** depicts endothelial cell lineage tracing in sham mice. **Fig. 7C** depicts endothelial cell lineage tracing in CKD mice. **Fig. 7D** depicts endothelial cell lineage tracing in CKD mice.

[0059] **Fig. 8** depicts the subject disposition. Note: "·" indicates the subjects with paired QCT measurements at baseline and Day 225. "*" indicates that the subject receiving placebo has elevated serum erythropoietin levels, suggesting off-protocol erythropoietin administration. "†" indicates a protocol violation. "‡" indicates the subject met stopping rule criteria for elevated blood pressure on Day 29; study treatment was discontinued, and rescue therapy was administered on Day 36, with continued follow-up. Subject was randomized in error with non-qualifying blood pressure, based on incomplete evaluation, at baseline .

[0060] **Fig. 9A** depicts the percentage of subjects in each treatment category with a greater than 2% femoral neck cortical bone increase. **Fig. 9B** depicts the percentage of subjects in each treatment group with any increase or decrease in trabecular bone mass in the lumbar spine.

[0061] **Fig. 10A** depicts the percentage of subjects in each treatment category for change in the total Agatston score. **Fig. 10B** depicts the percentage of subjects in each treatment category for change in the square root transformed total volume score.

[0062] **Fig. 11** depicts the percent change in the bone resorption biomarker CTX for subjects treated with placebo (PBO), or with an inhibitor of ActRII signaling at a dose of 0.3 mg/kg (0.3) or 0.5 mg/kg (0.5).

[0063] **Fig. 12A** demonstrates that CKD-3 mice treated with mActRIIA-Fc (CKD-3 R) at 10 mg/kg twice a week subcutaneously had no effect on inulin clearance. **Fig. 12B** demonstrates that CKD-3 mice treated with mActRIIA-Fc (CKD-3 R) at 10 mg/kg twice a week subcutaneously had no effect on BUN. **Fig. 12A** and **Fig. 12B** utilize A model of vascular calcification with ablative CKD as described in Section 9.4.1.1 was utilized to produce decreased kidney function similar to human CKD stage 3, referred to as CKD-3 (CKD-3 V for vehicle treated) with a 70% reduction in GFR compared to WT C57B6 mice (WT) estimated by inulin clearances and BUN. **Fig. 12C** demonstrates that Col4α5 deficient mice (Alport) at 200 days of age have reductions in inulin clearance equivalent to human stage 3-4 CKD. *$p<0.05$. **Fig. 12D** demonstrates that Col4α5 deficient mice (Alport) at 200 days of age have elevations in BUN equivalent to human stage 3-4 CKD. *$p<0.05$.

[0064] **Fig. 13** demonstrates that CKD increases Activin in the circulation and the kidney and aortic ActRIIA expression. "WT"=wildtype; "sham"=mice sham treated; "CKD-3 V"=mice treated with vehicle; "CKD-3 R"=mice treated with mActRIIA-Fc. **Fig. 13A** depicts the induction of circulating activin-A by CKD in a *ldlr-/-* high fat fed CKD-3 mouse model as described in Section 9.4.1.1. **Fig. 13B** depicts the induction of circulating activin-A by CKD in Alport's syndrome mice as described in as described in Section 9.4.1.1. **Fig. 13C** demonstrates inhibin betaA expression in mouse kidney and aorta (activin-A is formed of homodimers of the inhibin betaA gene). **Fig. 13D** demonstrates protein levels for inhibin betaA in kidney homogenates. 1, 2, and 3, refer to individual samples. **Fig. 13E** depicts immunohistochemistry of aortic ActRIIA. ActRIIA expression was detected in the aortas of wild type (WT) and sham operated $ldlr^{-/-}$ high fat fed mice (Sham), which was stimulated in CKD-3 mice treated with vehicle (CKD-3 V) or mActRIIA-Fc (CKD-3 mActRIIA-Fc). Scale bar is 20 μm. **Fig. 13F** demonstrates that there is no change in circulating follistatin levels in CKD-3 mice (treated with vehicle, CKD-3 V, or with mActRIIA-Fc, CKD-3 R) compared to WT and sham mice. *$p<0.05$, ***$p<0.005$. **Fig. 13G** demonstrates that there is no change in circulating follistatin like 3

(Fstl3) levels in CKD-3 mice (treated with vehicle, CKD-3 V, or with mActRIIA-Fc, CKD-3 R) compared to WT and sham mice. *p<0.05, ***p<0.005.

[0065] **Fig. 14** demonstrates CKD stimulated endothelial to mesenchymal transition. **Fig. 14A** depicts a diagram of mouse breeding strategy. ROSA26GNZ knock-in mouse (GNZ mouse) have sequences with stop codons, which prevent expression of nuclear localized GFP-LacZ reporter (GNZ). When breed with Tek-Cre mouse, which express Cre recombinase (Cre) in endothelial cells, Cre recombinase (Cre) deletes sequence flanked by *loxP* and all cells of endothelial origin produce GNZ reporter protein. **Fig. 14B** to **Fig. 14D** demonstrates double immunostaining of Tek-Cre/GNZ mouse aorta for endothelial cell marker CD31 and GFP with DAPI nuclear staining. Arrows point to double stained endothelial cells. In a Sham operated mouse (**Fig. 14B**) only endothelial cells demonstrate nuclear and cytoplasmic GFP staining. In CKD3 mice, (**Fig. 14C** and **Fig. 14D**) in addition to endothelial cells, cells in the aortic media and adventitia demonstrate GFP staining (arrowheads). Scale bar is 20 μm. **Fig. 14E** depicts the aortic mRNA levels of Snai 1, a transcription factor associated with EnMT (Medici, D., *et al.*, 2008. Molecular Biology of the Cell 19:4875-4887.), are increased in 75 day old Alport's mice compared to wild type (WT) mice. Alpha tubulin served as the loading control. 1 and 2 refer to individual samples.

[0066] **Fig. 15** depicts the effects of mActRIIA-Fc on vascular calcification in ldlr-/- high fat fed mice with CKD-3. **Fig. 15A** depicts Alizarin Red stained sections of proximal aortic atherosclerotic plaques from vehicle- (CKD-3 V) and mActRIIA-Fc-treated (CKD-3 mActRIIA-Fc) CKD-3 mice. **Fig. 15B** depicts aortic calcium levels in the groups of mice: wild type (wt); sham operated *ldlr-/-* high fat fed (sham); CKD-3 euthanasia at 22 weeks, the time of institution of treatment (CKD-3)); CKD-3 treated with vehicle from 22 to 28 weeks (CKD-3 V); CKD-3 treated with mActRIIA-Fc, 10 mg/kg subcutaneous twice weekly from 22 to 28 weeks (CKD-3 R). The boxes represent median (line in box) and interquartile ranges from 25^{th} to 75^{th} percentile. The error bars represent 1.5 fold of the interquartile range. Groups were compared using ANOVA Holm-Sidak method for multiple comparisons with $p<0.05$ as level for significant difference. * $p<0.02$, n for each group 8 – 12.

[0067] **Fig. 16** demonstrates the effects of decreased ActRIIA signaling on CKD stimulated cardiac disease. **Fig. 16A** demonstrates that CKD-3 in the *ldlr-/-* high fat fed mice increased heart weight, which was reversed in the mActRIIA-Fc treatment group, as compared to sham treated mice. CKD-3-L refers to CKD-3 mice treated with lanthanum carbonate. **Fig. 16B**

demonstrates that CKD-3 produced cardiac hypertrophy mainly left ventricular (middle), which was prevented in the mActRIIA-Fc treatment group (right), as compared to sham mice (left). Trichrome staining, scale bar 1mm. **Fig. 16C** depicts trichrome staining of left ventricular myocytes at higher magnification, demonstrating no evidence of cardiac fibrosis. Scale bar 20 μm.

[0068] **Fig. 17** demonstrates the CKD-3 effects on vascular stiffness in *ldlr-/-* high fat fed CKD-3 mice, and the absence of a Dkk1 neutralization effect. **Fig. 17A** depicts the pressure-diameter relationships performed as previously reported (Wagenseil, J.E., *et al.*, 2009. *Circulation Research* 104:1217-1224; Wagenseil, J.E., *et al.*, 2005. *AJP - Heart and Circulatory Physiology* 289:H1209-H1217.) of left common carotid arteries. **Fig. 17 B** depicts ascending aortas from wild- type (WT- 28 wks.), sham-operated (Sham - 28 wks.), CKD-3 (28 wks.), and CKD-3 treated with Dkk1 monoclonal antibody (Dkk1 mab – 28 wks.) mice. For **Fig. 17**, black represents WT or sham-operated mice, while open or grey represents CKD-3 mice. Values are presented as mean ± standard deviation. N = 5-6 mice for carotid arteries, 3-6 for ascending aortas.

[0069] **Fig. 18** demonstrates ActRIIA signaling in kidney and aorta. **Fig. 18A** depicts activin signaling determined by westerns of kidney (left) and aortic (right) homogenates from sham, CKD-3 vehicle- (CKD-3 V) and mActRIIA-Fc-treated (CKD-3 R) mice. Activin levels were increased in kidney homogenates but not in aortic homogenates, the Alk4 (AcvR1B) and Alk2 (AcvR1) type 1 receptors were present in kidney homogenates, but CKD-3 did not increase Alk4 phosphorylation. Alk4 and Alk1 (AcvRL1) were present in aortic homogenates. CKD-3 increased smad2/3 phosphorylation in kidneys but not aortas, and increased kidney Col1A1 levels. mActRIIA-Fc treatment decreased kidney phosphosmad2/3 and Col1A1 levels. In aortas, not only was there no effect of CKD-3 on phosphosmad2/3 levels, there was no effect on phosphoErk 1/2 levels. Runx2 levels were increased by CKD-3 and normalized by mActRIIA-Fc. **Fig. 18B** depicts a decrease of Klotho mRNA expression in kidney of CKD-3 mice and its correction by mActRIIA-Fc-treatment. **Fig. 18C** depicts Wnt signaling as marked by Dkk1 protein expression in westerns of kidney and aortic homogenates from sham, CKD-3 vehicle and mActRIIA-Fc treated mice (left), and the effect of CKD-3 and mActRIIA-Fc on plasma Dkk1 levels (right).

[0070] **Fig. 19** depicts plasma activin-A levels in patients with stage 3 CKD (n=30) versus healthy donors (n=10). Data were generated by measuring samples in duplicate using a

commercial ELISA kit (R&D systems, Minneapolis, MN). The coefficient of variation was 2.7% for the assay. *** $p<0.002$ Student t test.

[0071] **Fig. 20** depicts the levels of FGF-23 in wild type mice (WT), sham-treated mice (sham), CKD-3 mice (CKD-3 V), or CKD-3 mice treated with mActRIIA-Fc (CKD-3 R).

[0072] **Fig. 21A** depicts the level of circulating activin A in wild type mice (WT), sham-operated mice (Sham),CKD-3 mice treated with Vehicle (CKD-3 V), or CKD-3 mice treated mActRIIA-Fc (CKD-3 R). * indicates $p<0.005$. **Fig. 21B** depicts the osteoblast number/bone perimeter number/100 mm for sham-operated mice (Sham), CKD-3 mice treated with vehicle (CKD-3 V), or CKD-3 mice treated with mActRIIA-Fc (CKD-3 R). $p<0.05$ for CKD-3 V vs. CKD-3. **Fig. 21C** depicts the percentage of osteoblast surface/bone surface for sham-operated mice (Sham), CKD-3 mice treated with vehicle (CKD-3 V), or CKD-3 mice treated with mActRIIA-Fc (CKD-3 R). $p<0.05$ for CKD-3 V vs. CKD-3. **Fig. 21D** depicts the bone formation rate/osteoblast for sham-operated mice (Sham), CKD-3 mice treated with vehicle (CKD-3 V), or CKD-3 mice treated with mActRIIA-Fc (CKD-3 R). $p<0.05$ for CKD-3 V vs. CKD-3.

[0073] **Fig. 22A** depicts the subject disposition throughout the course of the study upon treatment with placebo or mActRIIA-Fc at the indicated doses. Each number on the y-axis represents an individual subject. **Fig. 22B** depicts the proportions of subjects with <15% progression in their abdominal aortal total Agatston score upon treatment with placebo or mActRIIA-Fc at the indicated doses. **Fig. 22C** depicts the proportions of subjects with >2% increase in femoral neck cortical BMD upon treatment with placebo or mActRIIA-Fc at the indicated doses.

[0074] **Fig. 23A** depicts a schematic diagram of the mouse fusion protein of the extracellular domain of ActRIIA and the Fc domain of IgG1 (mActRIIA-Fc). **Fig. 23B** depicts an experimental design of mActRIIA-Fc effects on the CKD- MBD in ldlr-/- high fat fed mice. Mice in four groups were fed the high fat diet beginning at 12 weeks (wks) of age. At 12 wks either sham operation (SO) or electrocautery cortical injury (EC) was performed. At 14 wks either SO or contralateral nephrectomy (NX) was performed. At 22 wks of life, vehicle treatment or mActRIIA-Fc , 10mg/kg subcutaneous twice weekly, was instituted. WT, wild type mice on chow diet for normal reference levels. Sham, sham operated ldlr-/- high fat fed mice; CKD-3, CKD-3 ldlr-/- high fat fed mice studied at 22 wks to establish levels of vascular calcium at the start of therapy; CKD-3 V, CKD-3 ldlr-/- high fat fed mice vehicle treated; CKD-3 R, CKD-3

ldlr-/- high fat fed mice mActRIIA-Fc treated; WT, Sham, CKD-3 V and CKD-3 R mice were euthanized at 28 weeks of age.

[0075] **Fig. 24A** depicts expression of ActRIIA in mouse aortas as determined by westerns for ActRIIA in aortic homogenates. **Fig. 24B** depicts immunoblot quantitation of **Fig. 24A**. For the quantitation, n=4, **p<0.0l. **Fig. 24C** depicts immunofluorescent detection of ActRIIA in the aortas of sham mice. **Fig. 24D** depicts immunofluorescent detection of ActRIIA in the aortas of CKD-3 mice. ActRIIA was expressed in aortic VSMC, but was not detected in endothelial cells. VSMC ActRIIA levels remained detectable in CKD compared to sham. CD31 (arrows) was used as an endothelial cell marker. Nuclei were stained by DAPI. Scale bar 20 μm.

[0076] **Fig. 25A** shows that CKD-3 causes increased mRNA expression of osteoblastic proteins (Runx2 and alkaline phosphatase (Alpl)), and decreased levels of aortic smooth muscle cell 22α (Tagln) which were all reversed by treatment with the mActRIIA-Fc. Aortic myocardin (Myocd) levels were decreased by CKD, but not affected by mActRIIA-Fc. **Fig. 25B** depicts westerns for proteins in aortic homogenates. Fig. 25C depicts immunoblot quantitation of the westerns of Fig. 25B. CKD causes decreased levels of aortic α- smooth muscle cell actin protein and increased levels of osteoblastic Runx2, which were reversed by treatment with mActRIIA-Fc , but myocardin levels were not changed. For the quantitation, n=4, **p<0.01.

[0077] **Fig. 26A** depicts the effects of CKD and the ActRIIA ligand trap on aortic calcification in ldlr-/- high fat fed mice with CKD-3 showing stained sections of proximal aortic atherosclerotic plaques with Alizarin Red from vehicle. **Fig. 26B** depicts the effects of CKD and the ActRIIA ligand trap on aortic calcification in ldlr-/- high fat fed mice with CKD-3 and treated with mActRIIA-Fc as shown by proximal aortic atherosclerotic plaques stained with Alizarin Red. Arrow head indicates calcium deposition in intima (i); m – media. Scale bar 100 μm. **Fig. 26C** depicts aortic calcium levels in the groups of mice: wild type (WT); sham operated ldlr-/- high fat fed (Sham); CKD-3 euthanasia at 22 weeks, the time of institution of treatment (CKD-3); CKD-3 treated with vehicle from 22 to 28 weeks (CKD-3 V); CKD-3 treated with mActRIIA-Fc , 10mg/kg subcutaneous twice weekly from 22 to 28 weeks (CKD-3 R). The boxes represent median (line in box) and interquartile ranges from 25th to 75th percentile. The error bars represent 1.5 fold of the interquartile range. Groups were compared using ANOVA Holm- Sidak method for multiple comparisons with p<0.05 as level for significant difference. *p<0.02; n for each goup 8-12.

[0078] **Fig. 27A** depicts the analysis of ActRIIA signaling by westerns of aortic homogenates from sham, CKD-3 vehicle and CKD-3 mActRIIA-Fc treated mice. Immunoblots of homogenates from two aortas of animals in each group. ActRIIA and Activin (inhibin β-A) levels were decreased in aortic homogenates from CKD-3 mice. The Alk4 (AcvR1B) and Alk1 (AcvRL1) type 1 receptors were present in aortic homogenates. CKD-3 decreased smad2/3 phosphorylation in aortas which was increased by mActRIIA-Fc treatment. CKD-3 decreased phospho- Erk 1/2 levels. Runx2 levels were increased by CKD-3 and normalized by mActRIIA-Fc treatment. **Fig. 27B** depicts p-Smad2/3 immunoblot quantitation, n=4, **p<0.01.

[0079] **Fig. 28A** depicts immunofluorescence microscopy of beta-catenin expression in the aortas of wild-type mice. **Fig. 28B** depicts immunofluorescence microscopy of beta-catenin expression in the aortas of a CKD mouse. There was no immunofluorescence for beta- catenin in the vascular smooth muscle cells. There was beta-catenin expression in the endothelium of aortas from CKD mice. Arrow heads, beta-catenin and CD31 colocalization in endothelial cells. Scale bar 20 μm. **Fig. 28C** depicts aortic Axin2 mRNA expression levels. **Fig. 28D** depicts the analysis of Wnt signaling as marked by Dkk1 protein expression in westerns of aortic homogenates from sham, CKD-3 V and CKD-3 R treated mice and by immunoblot quantitation, n=4. **Fig. 28E** depicts the effect of CKD-3V and mActRIIA-Fc on plasma Dkk1 levels. *p<0.05, **p<0.01.

[0080] **Fig. 29A** depicts the effects of mActRIIA-Fc treatment on renal klotho mRNA levels and demonstrates CKD-3 decreased klotho gene expression levels in kidney homogenates, and mActRIIA-Fc treatment significantly increased them compared to CKD- 3V. *p<0.05, **p<0.01, ***p<0.005. **Fig. 29B** depicts the analysis of ActRIIA signaling by westerns of kidney homogenates from sham, CKD-3 vehicle, and mActRIIA-Fc treated mice. All of the immunoblots are representative of homogenates from 3 or 4 kidneys. ActRIIA levels were not affected by CKD-3 or mActRIIA-Fc . Activin A (inhibin β-A) levels were increased in kidney homogenates of CKD-3 mice (quantitation is shown in Fig. 30A-B) and decreased by mActRIIA-Fc . The Alk4 (AcvR1B) and Alk2 (AcvR1) type 1 receptors were present in kidney homogenates, but CKD-3 V or CKD-3 R did not significantly affect Alk4 phosphorylation. CKD-3 increased renal smad2/3 phosphorylation and mActRIIA-Fc treatment decreased kidney phosphosmad2/3. **Fig. 29C** depicts immunoblot quantitation of Fig. 29B), *p<0.01.

[0081] **Fig. 30A** depicts trichrome staining of kidney sections from CKD-3V mActRIIA-Fc treated mice. **Fig. 30B** depicts trichrome staining of kidney sections from CKD-3V

mActRIIA-Fc treated mice. **Fig. 30C** depicts trichrome staining of kidney sections from CKD-3V mActRIIA-Fc treated mice. **Fig. 30D** depicts trichrome staining of kidney sections from CKD-3 mActRIIA-Fc treated mice. Areas of interstitial fibrosis marked by arrowheads. Kidneys of CKD-3 mActRIIA-Fc treated mice had decreased interstitial fibrosis. Scale bar 50 μm. *See* Fig. 35 for marking of whole kidney coronal sections as to where the photomicrograph sections were taken. **Fig. 30E** depicts the effects of mActRIIA-Fc on urinary protein. There was significant proteinuria in the CKD-3V mice, which was decreased by mActRIIA-Fc treatment, *$p<0.05$.

[0082] **Fig. 31A** depicts induction of circulating activin-A by CKD in atherosclerotic ldlr-/- high fat fed CKD- 3 mice. **Fig. 31B** depicts induction of circulating activin-A by CKD in Alport's syndrome mice as described herein. **Fig. 31C** depicts inhibin betaA (Inhba) mRNA expression in mouse kidney (activin-A is formed of homodimers of inhibin betaA). **Fig. 31D** depicts westerns for inhibin β-A in kidney homogenates. **Fig. 31E** depicts immunoblot quantitation of **Fig. 31D**, n=6 for the immunoblot quantitation; *$p<0.05$, **$p<0.01$, ***$p<0.005$.

[0083] **Fig. 32A** shows kidney InhbA immunostaining in: sham. **Fig. 32B** shows kidney InhbA immunostaining in CKD-3 Vehicle mice. In Sham mice occasional peritubular interstitial cells express activin-A, but in CKD-3 mice many more peritubular interstitial cells are positive for activin-A at varying levels of intensity. Scale bar 50 μm.

[0084] **Fig. 33A** and **Fig. 33B** depict chronic kidney disease in mice analogous to human stage 3 kidney disease. CKD was staged in the mouse models in a manner analogous to the staging of human CKD. The model of vascular calcification with ablative CKD as described in methods (Section 9.10.1) was used to produce decreased kidney function similar to human CKD stage 3, referred to as CKD-3 (CKD-3 V for vehicle treated) with a 70% reduction in GFR compared to WT C57B6 mice (WT) estimated by inulin clearances and BUN. Treatment of CKD-3 mice with mActRIIA-Fc (CKD-3 R), 10 mg/kg twice weekly subcutaneously, had no effect on inulin clearance or BUN.

[0085] **Fig. 34** depicts a diagrammatic representation of ActRIIA signaling from Tsuchida et al (Cell Comm. and Signaling, 2009). Activin, Myostatin, and GDF11 binding to the type II receptors, ActRIIA (primary for activin) and ActRIIB (primary for myostatin and GDF11) activate signal transduction pathways including the canonical pathway through smad 2,3.

[0086] **Fig. 35A** and **Fig. 35B** show kidneys (kidney trichrome staining) of CKD-3 vehicle treated mice. **Fig. 35C** and **Fig. 35D** show kidneys (kidney trichrome staining) of CKD-3 mActRIIA-Fc treated mice. Scale bar 1mm. The cortical surface scars from the electrocautery injury are marked by arrowheads. The arrows designate the point from which the high power photomicrographs in Fig. 36 were taken. Scale bar 1 mm.

[0087] **Fig. 36A** depicts an absence of change in circulating follistatin levels in CKD-3 mice compared to WT and sham mice. **Fig. 36B** depicts an absence of change in circulating follistatin like 3 (Fstl3) levels in CKD-3 mice compared to WT and sham mice.

[0088] **Fig. 37** depicts the levels of Activin Receptor Interacting Proteins (Arip1 and Arip2) in tissues of CKD-3 mice. Arip1 is strongly expressed in the remnant kidney of CKD-3 mice, more that twofold greater than Arip2 expression. Arip1 is expressed at low levels in the aortas of CKD-3 mice, while Arip2 expression in the aorta is twice that of Arip1.

8. DETAILED DESCRIPTION

8.1 OVERVIEW

[0089] Provided herein, in one aspect, is a method for the treatment and/or prevention of cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, and/or cardiovascular disease associated with and/or resulting from renal disease, elevated levels of arterial stiffness (*e.g.*, as indicated by decreased vascular compliance), and/or left ventricular hypertrophy (LVH), including cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness and/or LVH, wherein the method comprises administering an inhibitor of ActRII signaling (*e.g.*, an activin ligand trap) to a subject in need of treatment and/or prevention thereof. In certain embodiments, the subject is a renal subject. The inhibitor of ActRII signaling can be an inhibitor of ActRIIA signaling and / or ActRIIB signaling.

[0090] In particular, provided herein are methods of treating and/or preventing cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, cardiovascular disease associated with and/or resulting from renal disease, elevated levels of arterial stiffness, and/or left ventricular hypertrophy (LVH) by using the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha, as an indicator of the patient population, as an indicator of responsiveness of a subject to the treatment and/or prevention with an ActRII

signaling inhibitor, as an indicator of efficacy of the treatment with an ActRII signaling inhibitor, or as an indicator of appropriate dosage for the treatment with an ActRII signaling inhibitor. The levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha can also be utilized to identify diseases and/or conditions for treatment and/or prevention with an ActRII signaling inhibitor. An ActRII signaling inhibitor used in the methods described herein can be an inhibitor of ActRIIA signaling and/or ActRIIB signaling, such as any of the inhibitors described herein or known in the art. In a preferred embodiment, an ActRII signaling inhibitor is a humanized fusion-protein consisting of the extracellular domain of ActRIIA and the human IgG1 Fc domain ("ActRIIA-Fc," *e.g.*, SEQ ID NO:7).

[0091] The methods provided herein are based, in part, on the discovery that the levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and Osterix are elevated, and the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, and Sm22-alpha are decreased in a mouse model of chronic kidney disease-induced vascular calcification as compared to a wildtype mouse. Further, without being limited by theory, ligand trapping by mActRIIA-Fc reduces vascular calcification in a chronic kidney disease mouse model (ldlr-/- mice, high fat diet). As shown in the Examples presented herein (*See* Section 9), treatment of chronic kidney disease mice with mActRIIA-Fc reduced vascular calcification, reduced aortic calcium levels, reduced phosphosmad3 levels, reduced urinary protein levels, reduced phosphosmad2 levels, reduced Dkk1 levels, reduced Runx2 levels, reduced Alp levels, reduced BSAP levels, reduced CTX levels, and reduced Osterix levels, and elevated Klotho levels, elevated alpha-SMA levels, elevated Axin2 levels and elevated Sm22-alpha levels. Furthermore, the methods provided herein are based, in part, on the discovery that the decrease in vascular calcification in chronic kidney disease mice upon treatment with mActRIIA-Fc is correlated with a decrease in Dkk1, phosphosmad2, Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix levels, and an increase in Klotho, alpha-SMA, Axin2, and/or Sm22-alpha levels (*See* Examples, Section 9). Taken together, the data presented herein indicate that Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha levels can identify which subjects may respond to ActRIIA-Fc and/or can be used to monitor clinical response to an ActRII signaling inhibitor and/or select target

patient populations. The data presented herein also indicate that ActRIIA-Fc (*e.g.*, mActRIIA-Fc, or ActRIIA-hFc such as SEQ ID NO:7) is useful in treating vascular calcification associated with chronic kidney disease. Additionally, the data presented herein also indicate that ActRIIA-Fc (*e.g.*, mActRIIA-Fc, or ActRIIA-hFc such as SEQ ID NO:7) is useful in treating diseases associated with elevated levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin) Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix, or diseases associated with decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha. Finally, the data presented herein also indicate that ActRIIA-Fc (*e.g.*, mActRIIA-Fc, or hActRIIA-hFc such as SEQ ID NO:7) is useful in decreasing calcium deposits in aortic atheroma, reducing aortic calcium levels, reversing the CKD-induced increase in heart weight, and decreasing renal fibrosis, and accordingly, is useful in treating cardiovascular disease.

[0092] The findings described herein, illustrated in the Examples (*See* Section 9), indicate that detection of the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, or Sm22-alpha can be used (i) as a marker (*e.g.*, serum biomarker) of the extent of vascular calcification in a subject, (ii) as a marker to measure the subject's response to an ActRII signaling inhibitor (such as ActRIIA-Fc), or (iii) to evaluate the pharmacodynamic effects of an ActRII signaling inhibitor (such as ActRIIA-Fc) in a subject following treatment, wherein the subject is a subject with cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, and/or cardiovascular disease associated with and/or resulting from renal disease. Thus, in certain embodiments, Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, Axin2, and/or Sm22-alpha can be used in the methods described herein as an indicator of efficacy of ActRIIA-Fc (*e.g.*, ActRIIA-hFc such as SEQ ID NO:7) treatment and/or as an indicator of absence of response to the treatment with ActRIIA-Fc (*e.g.*, ActRIIA-hFc such as SEQ ID NO:7). In addition, as described herein, Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha can be used as a reliable molecular marker to evaluate time-course treatment efficacy of ActRIIA-Fc (*e.g.*, ActRIIA-hFc such as SEQ ID NO:7). Further, in a specific embodiment, provided herein is a method which comprises detection of the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin

(*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the blood (*e.g.*, detecting abnormal expression in a disease associated with impaired vascular smooth muscle cell function), and administration of an ActRII signaling inhibitor, such as ActRIIA-Fc, in a dose dependent on the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha.

[0093] In certain embodiments, the ActRII signaling inhibitor is Sotatercept (SEQ ID NO: 7; *see, e.g.*, Section 9). Sotatercept is an activin ligand trap that is useful in the methods described herein (*see* Section 8).

8.2 ABBREVIATIONS

[0094] As used herein, "Snai 1" or "Snai1" refer to snail homolog 1. *See*, for example, Twigg and Wilkie, 1999, Hum Genet. 105(4):320-326 GenBank™ accession number NM_005985.3 provides an exemplary human Snai1 nucleic acid sequence. GenBank™ accession number NP_005976.2 provides an exemplary human Snai1 amino acid sequence.

[0095] As used herein, "phosphosmad3" refers to phosphorylated mothers against decapentaplegic homolog 3. *See*, for example, Matsuzaki, 2013, Cytokine Growth Factor Rev, 24(4):385-399. GenBank™ accession number NM_005902.3 provides an exemplary human phosphosmad3 nucleic acid sequence. GenBank™ accession number NP_005893.1 provides an exemplary human phosphosmad3 amino acid sequence.

[0096] As used herein, "phosphosmad2" refers to phosphorylated mothers against decapentaplegic homolog 2. *See*, for example, Matsuzaki, 2013, Cytokine Growth Factor Rev, 24(4):385-399. GenBank™ accession number NM_001003652.3 provides an exemplary human phosphosmad2 nucleic acid sequence. GenBank™ accession number NP_001003652.1 provides an exemplary human phosphosmad2 amino acid sequence.

[0097] As used herein, "Dkk1" refers to dickkopf-related protein 1. *See*, for example, Fang, Y., *et al.*, 2014. J Am Soc Nephrol 25:1760-1763. GenBank™ accession number NM_012242.2 provides an exemplary human Dkk1 nucleic acid sequence. GenBank™ accession number NP_036374.1 provides an exemplary human Dkk1 amino acid sequence.

[0098] As used herein, "col1a1" refers to collagen type 1, alpha 1. *See*, for example, Korkko *et al.*, 1998, Am. J. Hum. Genet., 62:98-110. GenBank™ accession number

XM_005257059.2 provides an exemplary human col1a1 nucleic acid sequence. GenBank™ accession number XP_005257116.2 provides an exemplary human col1a1 amino acid sequence.

[0099] As used herein, "Runx2" refers to runt-related transcription factor 2. *See*, for example, Komori, 2010, Adv Exp Med Biol, 658:43-49. GenBank™ accession number NM_001145920.2 provides an exemplary human Runx2 nucleic acid sequence. GenBank™ accession number NP_001139392.1 provides an exemplary human Runx2 amino acid sequence.

[00100] As used herein, "Alp" or "Alpl" refers to alkaline phosphatase. As used herein, "BSAP" refers to bone-specific alkaline phosphatase. *See*, for example, Martins *et al.*, 2013, Bone, 56(2):390-397. GenBank™ accession number NM_000478.4 provides an exemplary human Alp nucleic acid sequence. GenBank™ accession number NP_000469.3 provides an exemplary human Alp amino acid sequence.

[00101] As used herein, "CTX" refers to C-terminal telopeptides type I collagen. *See*, for example, Rosen et al, 2000, Calcif Tissue Int. 66(2): 100-103.

[00102] As used herein, "Osterix" refers to Osterix, also known as the Sp7 transcription factor. *See*, for example, Cao *et al.*, 2005, Cancer Res. 65(4):1124-1128. GenBank™ accession number NM_001173467.2 provides an exemplary human Osterix nucleic acid sequence. GenBank™ accession number NP_001166938.1 provides an exemplary human Osterix amino acid sequence.

[00103] As used herein, "Klotho" refers to Klotho. *See*, for example, Matsumura *et al.* 1998, Biochem Biophys Res Commun, 242:626-630. GenBank™ accession number NM_004795.3 provides an exemplary human Klotho nucleic acid sequence. GenBank™ accession number NP_004786.2 provides an exemplary human Klotho amino acid sequence.

[00104] As used herein, "alpha-SMA" or "αSMA" or "α-SMA" refers to alpha smooth muscle actin. *See*, for example, Nowak *et al.*, 1999, Nat. Genet. 23:208-212. GenBank™ accession number NM_001100.3 provides an exemplary human alpha-SMA nucleic acid sequence. GenBank™ accession number NP_001091.1 provides an exemplary human alpha-SMA amino acid sequence.

[00105] As used herein, "MYOCD" refers to myocardin. *See*, for example, Imamura *et al.*, 2010, Gene, 464:1-10. GenBank™ accession number NM_001146312.2 provides an exemplary human MYOCD nucleic acid sequence. GenBank™ accession number NP_001139784.1 provides an exemplary human MYOCD amino acid sequence.

[00106] As used herein, "Sm22-alpha" or "Sm22α" or "Sm22-α" refers to smooth muscle protein 22 alpha, also known as "transgelin", "Tagln", or "Tagln1". *See*, for example, Camoretti-Mercado, 1998, Genomics, 49:452-457. GenBank™ accession number NM_001001522.1 provides an exemplary human Sm22-alpha nucleic acid sequence. GenBank™ accession number NP_001001522.1 provides an exemplary human Sm22-alpha amino acid sequence.

[00107] As used herein, "ActRII" refers to activin receptor type II. As used herein, "ActRIIA" refers to activin receptor type IIA. *See*, for example, Mathews and Vale, 1991, Cell 65:973-982. GenBank™ accession number NM_001278579.1 provides an exemplary human ActRIIA nucleic acid sequence. GenBank™ accession number NP_001265508.1 provides an exemplary human ActRIIA amino acid sequence. As used herein, "ActRIIB" refers to activin receptor type IIB. *See*, for example, Attisano *et al.*, 1992, Cell 68: 97-108. GenBank™ accession number NM_001106.3 provides an exemplary human ActRIIB nucleic acid sequence. GenBank™ accession number NP_001097.2 provides an exemplary human ActRIIB amino acid sequence.

[00108] As used herein, "Axin2" refers to axis inhibitor 2. GenBank™ accession number NM_004655.3 provides an exemplary human Axin2 nucleic acid sequence. GenBank™ accession number NP_004646.3 provides an exemplary human amino acid sequence.

[00109] As used herein, "mActRIIA-Fc" or "ActRIIA-mFc" refers to a mouse activin type IIA receptor-IgG1 fusion protein. *See*, for example, U.S. Patent No. 8,173,601. As used herein, "mActRIIB-Fc" or "ActRIIB-mFc" refers to a mouse activin type IIB receptor-IgG1 fusion protein. *See*, for example, U.S. Patent No. 8,173,601. As used herein, "hActRIIA-Fc" or "ActRIIA-hFc" refers to a human activin type IIA receptor-IgG1 fusion protein. *See*, for example, U.S. Patent No. 8,173,601. As used herein, "hActRIIB-Fc" or "ActRIIB-hFc" refers to a human activin type IIB receptor-IgG1 fusion protein. *See*, for example, U.S. Patent No. 8,173,601.

[00110] As used herein, "LVH" refers to left ventricular hypertrophy.

8.3 METHODS OF TREATMENT AND/OR PREVENTION

8.3.1 CARDIOVASCULAR DISEASE AND/OR VASCULAR CALCIFICATION

[00111] In certain embodiments, provided herein are methods for treating and/or preventing cardiovascular disease, vascular calcification, cardiovascular disease associated with

and/or resulting from vascular calcification, cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness, elevated levels of arterial stiffness, left ventricular hypertrophy, cardiovascular disease associated with and/or resulting from left ventricular hypertrophy, and/or cardiovascular disease associated with and/or resulting from renal disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap). In certain embodiments, the subject is a renal subject. In certain other embodiments, the subject has been diagnosed as having chronic kidney disease-mineral bone disorder. In certain other embodiments, the subject has not been diagnosed as having chronic kidney disease-mineral bone disorder.

[00112] In certain embodiments, the subject has elevated levels and/or activity of Runx2 as compared to levels and/or activity of Runx2 in a reference population, elevated levels and/or activity of Alp as compared to levels and/or activity of Alp in a reference population, elevated levels and/or activity of Snai1 as compared to levels and/or activity of Snai1 in a reference population, elevated levels and/or activity of phosphosmad2 as compared to levels and/or activity of phosphosmad2 in a reference population, elevated levels and/or activity of phosphosmad3 as compared to levels and/or activity of phosphosmad3 in a reference population, elevated levels and/or activity of urinary protein as compared to levels and/or activity of urinary protein in a reference population, elevated levels and/or activity of Dkk1 as compared to levels and/or activity of Dkk1 in a reference population, elevated levels and/or activity of activin (*e.g.*, free activin) as compared to levels and/or activity of activin (*e.g.*, free activin) in a reference population, elevated levels and/or activity of col1a1 as compared to levels and/or activity of col1a1 in a reference population, elevated levels and/or activity of BSAP as compared to levels and/or activity of BSAP in a reference population, elevated levels and/or activity of CTX as compared to levels and/or activity of CTX in a reference population, elevated levels and/or activity of Osterix as compared to levels and/or activity of Osterix in a reference population, decreased levels and/or activity of Klotho as compared to levels and/or activity of Klotho in a reference population, decreased levels and/or activity of alpha-SMA as compared to levels and/or activity of alpha-SMA in a reference population, decreased levels and/or activity of MYOCD as compared to levels and/or activity of MYOCD in a reference population, decreased levels and/or activity of Sm22-alpha as compared to levels and/or activity of Sm22-alpha in a reference population; and/or decreased levels and/or activity of ActRIIA as compared to levels and/or activity of ActRIIA in a reference population.

[00113] In certain embodiments, the subject has elevated levels and/or activity of Runx2 as compared to prior levels and/or activity of Runx2 in the subject, elevated levels and/or activity of Alp as compared to prior levels and/or activity of Alp in the subject, elevated levels and/or activity of Snai1 as compared to prior levels and/or activity of Snai1 in the subject, elevated levels and/or activity of phosphosmad2 as compared to prior levels and/or activity of phosphosmad2 in the subject, elevated levels and/or activity of phosphosmad3 as compared to prior levels and/or activity of phosphosmad3 in the subject, elevated levels and/or activity of urinary protein as compared to prior levels and/or activity of urinary protein in the subject, elevated levels and/or activity of Dkk1 as compared to prior levels and/or activity of Dkk1 in the subject, elevated levels and/or activity of activin (*e.g.*, free activin) as compared to prior levels and/or activity of activin (*e.g.*, free activin) in the subject, elevated levels and/or activity of col1a1 as compared to prior levels and/or activity of col1a1 in the subject, elevated levels and/or activity of BSAP as compared to prior levels and/or activity of BSAP in the subject, elevated levels and/or activity of CTX as compared to prior levels and/or activity of CTX in the subject, elevated levels and/or activity of Osterix as compared to prior levels and/or activity of Osterix in the subject, decreased levels and/or activity of Klotho as compared to prior levels and/or activity of Klotho in the subject, decreased levels and/or activity of alpha-SMA as compared to prior levels and/or activity of alpha-SMA in the subject, decreased levels and/or activity of MYOCD as compared to prior levels and/or activity of MYOCD in the subject, decreased levels and/or activity of ActRIIA as compared to prior levels and/or activity of ActRIIA in the subject, and/or decreased levels and/or activity of Sm22-alpha as compared to prior levels and/or activity of Sm22-alpha in the subject. In certain embodiments, the prior levels and/or activity of Runx2 in the subject, the prior levels and/or activity of Alp in the subject, the prior levels and/or activity of Snai1 in the subject, the prior levels and/or activity of phosphosmad2 in the subject, the prior levels and/or activity of phosphosmad3 in the subject, the prior levels and/or activity of urinary protein in the subject, the prior levels and/or activity of Dkk1 in the subject, the prior levels and/or activity of activin (*e.g.*, free activin) in the subject, the prior levels and/or activity of col1a1 in the subject, the prior levels and/or activity of BSAP in the subject, the prior levels and/or activity of CTX in the subject, the prior levels and/or activity of Osterix in the subject, the prior levels and/or activity of Klotho in the subject, the prior levels and/or activity of alpha-SMA in the subject, the prior levels and/or activity of MYOCD in the subject, the prior levels and/or activity of ActRIIA in the subject, and/or the prior levels and/or activity of Sm22-alpha in the

subject are the respective levels 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, or 48 months before the onset of symptoms or the diagnosis of (i) cardiovascular disease; (ii) vascular calcification; (iii) cardiovascular disease associated with and/or resulting from vascular calcification; (iv) cardiovascular disease associated with and/or resulting from renal disease; (v) elevated levels of arterial stiffness; and/or left ventricular hypertrophy (LVH).

[00114] In certain embodiments, the subject is treated in accordance with the methods as described in 8.3.4. In certain embodiments, the vascular calcification in the subject is analyzed by measuring Agatston scores as described in Section 8.6.

[00115] In certain embodiments, a subject to be treated with the methods provided herein has the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha as described in Section 8.6. Thus, in certain specific embodiments, a method provided herein comprises (i) selecting a subject based on the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, and/or Sm22-alpha as described in Section 8.6; and (ii) administering a pharmaceutically effective dose of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap). In a specific embodiment, the subject has cardiovascular disease. In a specific embodiment, the subject has vascular calcification. In a specific embodiment, the subject has cardiovascular disease associated with and/or resulting from vascular calcification. In a specific embodiment, the subject has cardiovascular disease associated with and/or resulting from renal disease. In a specific embodiment, the subject has renal disease. In certain other embodiments, the subject has been diagnosed as having chronic kidney disease-mineral bone disorder. In certain other embodiments, the subject has not been diagnosed as having chronic kidney disease-mineral bone disorder. In certain embodiments, the vascular calcification in the subject is analyzed by measuring Agatston scores as described in Section 8.6. In specific embodiments, the subject has been diagnosed with elevated levels of arterial stiffness. In a specific embodiment, the subject has been diagnosed with LVH. In a specific embodiment, the subject has cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness. In a specific embodiment, the subject has cardiovascular disease associated with and/or resulting from LVH.

[00116] In certain embodiments, the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA,

MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha are determined as described in Section 8.6. In certain embodiments, the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha are the protein level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha, respectively. In certain embodiments, the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha are the mRNA level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha, respectively. In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, and/or ActRIIA, Axin2, alpha-SMA levels are in a tissue. In certain embodiments, the tissue is aorta. In certain embodiments, the tissue is kidney. In certain embodiments, the tissue is bone. In certain embodiments, the tissue is serum. In a preferred embodiment, the Runx2, Dkk1, Alp, osterix, sm22-alpha, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2 levels and/or activity are in aorta. In a preferred embodiment, the phosphosmad2, phosphosmad3, urinary protein, ActRIIA, Axin2, and col1a1 levels and/or activity are in kidney. In a preferred embodiment, the activin levels and/or activity are in serum. In certain embodiments, the levels of activin are elevated in the subject with respect to a reference population. In certain embodiments, the levels of follistatin in the subject are about equal to the levels of follistatin in a reference population.

[00117] In certain embodiments, the reference population is a population as described in Section 8.6. In certain embodiments, the subject is a subject as described in Section 8.4.

[00118] In certain embodiments, the ActRII signaling inhibitor is ActRIIA-Fc such as ActRIIA-hFc (*e.g.*, SEQ ID NO:7). In certain embodiments, the ActRII signaling inhibitor is an ActRII signaling inhibitor as described in Section 8.5.

[00119] In certain embodiments, the pharmaceutically effective dose is a dose as described in Section 8.7. In certain embodiments, the pharmaceutically effective dose is an initial dose. In certain embodiments, the pharmaceutically effectively dose is administered at a frequency as

described in Section 8.7. In certain embodiments, the pharmaceutically effective dose is administered as described in Section 8.7.

[00120] In certain embodiments, the methods provided herein are utilized in combination with a second pharmaceutically active agent, as described in Section 8.8.

[00121] In certain embodiments, "treat," "treatment," or "treating," in the context of cardiovascular disease or chronic kidney disease, includes amelioration of at least one symptom of cardiovascular disease or chronic kidney disease, respectively.

[00122] As will be recognized by one of skill in the art, levels and/or activity of one or more of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, Sm22-alpha, phosphosmad3, and urinary protein can be compared respectively to the level and/or activity in a corresponding reference population. Thus, in certain embodiments, the level and/or activity one of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of two of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of three of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of three of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of four of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of five of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of six of Runx2, Alp, Snai1, phosphosmad2,

Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of seven of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of eight of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of nine of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of ten of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of eleven of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of twelve of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of thirteen of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of fourteen of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of fifteen of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha,

phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activityof sixteen of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of seventeen of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population. In certain embodiments, the level and/or activity of each of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein is compared to its level and/or activity in a corresponding reference population.

8.3.2 DISEASES ASSOCIATED WITH SNAI1, PHOSPHOSMAD2, DKK1, COL1A1, ACTIVIN (*E.G.*, FREE ACTIVIN), RUNX2, ALP, BSAP, CTX, OSTERIX, KLOTHO, ALPHA-SMA, MYOCD, PHOSPHOSMAD3, URINARY PROTEIN, ACTRIIA, AXIN2, AND/OR SM22-ALPHA

[00123] In certain embodiments, provided herein are methods for treating and/or preventing one or more diseases associated with Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap).

[00124] In certain embodiments, the subject is a subject as described in Section 8.4.

[00125] In certain embodiments, the ActRII signaling inhibitor is ActRIIA-Fc such as ActRIIA-hFc (*e.g.*, SEQ ID NO:7). In certain embodiments, the ActRII signaling inhibitor is an ActRII signaling inhibitor as described in Section 8.5.

[00126] In certain embodiments, the pharmaceutically effective dose is a dose as described in Section 8.7. In certain embodiments, the pharmaceutically effective dose is an initial dose. In certain embodiments, the pharmaceutically effectively dose is administered at a frequency as described in Section 8.7. In certain embodiments, the pharmaceutically effective dose is administered as described in Section 8.7. In certain embodiments, the pharmaceutically effective dose is adjusted depending on the level and/or activity of certain biomarkers as described herein.

[00127] In certain embodiments, the methods provided herein are utilized in combination with a second pharmaceutically active agent, as described in Section 8.8.

8.3.3 CHRONIC KIDNEY DISEASE-MINERAL/BONE DISORDER

[00128] In certain embodiments, provided herein are methods for treating chronic kidney disease-mineral bone disorder in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap). In certain embodiments, provided herein are methods for reducing bone resorption in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor. In certain embodiments, the subject has cardiovascular disease. In certain embodiments, the subject has vascular calcification. In certain embodiments, the subject has cardiovascular disease associated with and/or resulting from vascular calcification. In certain embodiments, the subject has cardiovascular disease associated with and/or resulting from renal disease. In certain embodiments, the subject is a renal subject. In certain other embodiments, the subject has been diagnosed as having chronic kidney disease-mineral bone disorder. In certain other embodiments, the subject has not been diagnosed as having chronic kidney disease-mineral bone disorder.

[00129] In certain embodiments, the subject has elevated levels and/or activity of Runx2 as compared to levels and/or activity of Runx2 in a reference population, elevated levels and/or activity of Alp as compared to levels and/or activity of Alp in a reference population, elevated levels and/or activity of Snai1 as compared to levels and/or activity of Snai1 in a reference population, elevated levels and/or activity of phosphosmad2 as compared to levels and/or activity of phosphosmad2 in a reference population, elevated levels and/or activity of phosphosmad3 as compared to levels and/or activity of phosphosmad3 in a reference population, elevated levels and/or activity of urinary protein as compared to levels and/or activity of urinary protein in a reference population, elevated levels and/or activity of Dkk1 as compared to levels and/or activity of Dkk1 in a reference population, elevated levels and/or activity of col1a1 as compared to levels and/or activity of col1a1 in a reference population, elevated levels and/or activity of activin *(e.g.*, free activin) as compared to levels and/or activity of activin *(e.g.*, free activin) in a reference population, elevated levels and/or activity of BSAP as compared to levels and/or activity of BSAP in a reference population, elevated levels and/or activity of CTX as compared to levels and/or activity of CTX in a reference population, elevated levels and/or activity of Osterix as compared to levels and/or activity of Osterix in a reference population,

decreased levels and/or activity of Klotho as compared to levels and/or activity of Klotho in a reference population, decreased levels and/or activity of alpha-SMA as compared to levels and/or activity of alpha-SMA in a reference population, decreased levels and/or activity of MYOCD as compared to levels and/or activity of MYOCD in a reference population, decreased levels and/or activity of ActRIIA as compared to levels and/or activity of ActRIIA in a reference population, and/or decreased levels and/or activity of Sm22-alpha as compared to levels and/or activity of Sm22-alpha in a reference population. In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, phosphosmad3, urinary protein, ActRIIA, and/or alpha-SMA levels are in a tissue. In certain embodiments, the tissue is aorta. In certain embodiments, the tissue is kidney. In certain embodiments, the tissue is bone. In certain embodiments, the tissue is serum. In a preferred embodiment, the Runx2, Dkk1, Alp, osterix, sm22-alpha, Klotho, alpha-SMA, MYOCD, and/or ActRIIA levels and/or activity are in aorta. In a preferred embodiment, the phosphosmad2, phosphosmad3, urinary protein, and col1a1, and/or ActRIIA levels and/or activity are in kidney. In a preferred embodiment, the activin levels and/or activity are in serum. In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, ActRIIA, and/or alpha-SMA levels are in aorta.

[00130] In certain embodiments, the subject has elevated levels and/or activity of Runx2 as compared to prior levels and/or activity of Runx2 in the subject, elevated levels and/or activity of Alp as compared to prior levels and/or activity of Alp in the subject, elevated levels and/or activity of Snai1 as compared to prior levels and/or activity of Snai1 in the subject, elevated levels and/or activity of phosphosmad2 as compared to prior levels and/or activity of phosphosmad2 in the subject, elevated levels and/or activity of phosphosmad3 as compared to prior levels and/or activity of phosphosmad3 in the subject, elevated levels and/or activity of urinary protein as compared to prior levels and/or activity of urinary protein in the subject, elevated levels and/or activity of Dkk1 as compared to prior levels and/or activity of Dkk1 in the subject, elevated levels and/or activity of activin (*e.g.*, free activin) as compared to prior levels and/or activity of activin (*e.g.*, free activin) in the subject, elevated levels and/or activity of col1a1 as compared to prior levels and/or activity of col1a1 in the subject, elevated levels and/or activity of BSAP as compared to prior levels and/or activity of BSAP in the subject, elevated levels and/or activity of CTX as compared to prior levels and/or activity of CTX in the subject,

elevated levels and/or activity of Osterix as compared to prior levels and/or activity of Osterix in the subject, decreased levels and/or activity of Klotho as compared to prior levels and/or activity of Klotho in the subject, decreased levels and/or activity of alpha-SMA as compared to prior levels and/or activity of alpha-SMA in the subject, decreased levels and/or activity of MYOCD as compared to prior levels and/or activity of MYOCD in the subject, decreased levels and/or activity of ActRIIA as compared to prior levels and/or activity of ActRIIA in the subject, and/or decreased levels and/or activity of Sm22-alpha as compared to prior levels and/or activity of Sm22-alpha in the subject. In certain embodiments, the prior levels and/or activity of Runx2 in the subject, the prior levels and/or activity of Alp in the subject, the prior levels and/or activity of Snai1 in the subject, the prior levels and/or activity of phosphosmad2 in the subject, the prior levels and/or activity of phosphosmad3 in the subject, the prior levels and/or activity of urinary protein in the subject, the prior levels and/or activity of Dkk1 in the subject, the prior levels and/or activity of activin (*e.g.*, free activin) in the subject, the prior levels and/or activity of col1a1 in the subject, the prior levels and/or activity of BSAP in the subject, the prior levels and/or activity of CTX in the subject, the prior levels and/or activity of Osterix in the subject, the prior levels and/or activity of Klotho in the subject, the prior levels and/or activity of alpha-SMA in the subject, the prior levels and/or activity of MYOCD in the subject, the prior levels and/or activity of ActRIIA in the subject, and/or the prior levels and/or activity of Sm22-alpha in the subject are the respective levels 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, or 48 months before the onset of symptoms or the diagnosis of CKD-MBD.

[00131] In certain embodiments, the subject is treated in accordance with the methods as described in Section 8.3.4. In certain embodiments, the vascular calcification in the subject is analyzed by measuring Agatston scores as described in Section 8.6.

[00132] In certain embodiments, a subject to be treated with the methods provided herein has the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha as described in Section 8.6. Thus, in certain specific embodiments, a method provided herein comprises (i) selecting a subject based on the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, and/or Sm22-alpha as described in Section 8.6; and (ii) administering a pharmaceutically effective dose of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap). In

a specific embodiment, the subject has cardiovascular disease. In a specific embodiment, the subject has vascular calcification. In a specific embodiment, the subject has cardiovascular disease associated with and/or resulting from vascular calcification. In a specific embodiment, the subject has cardiovascular disease associated with and/or resulting from renal disease. In a specific embodiment, the subject has renal disease. In certain other embodiments, the subject has been diagnosed as having chronic kidney disease-mineral bone disorder. In certain other embodiments, the subject has not been diagnosed as having chronic kidney disease-mineral bone disorder. In a specific embodiment, the subject has elevated levels of arterial stiffness. In a specific embodiment, the subject has cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness. In a specific embodiment, the subject has LVH. In a specific embodiment, the subject has cardiovascular disease associated with and/or resulting from LVH.

[00133] In certain embodiments, the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha are determined as described in Section 8.6. In certain embodiments, the elevated levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix are as described in Section 8.6. In certain embodiments, the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha are as described in Section 8.6. In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, phosphosmad3, urinary protein, ActRIIA, and/or alpha-SMA levels are in a tissue. In certain embodiments, the tissue is aorta. In certain embodiments, the tissue is kidney. In certain embodiments, the tissue is bone. In certain embodiments, the tissue is serum. In a preferred embodiment, the Runx2, Dkk1, Alp, osterix, sm22-alpha, Klotho, alpha-SMA, ActRIIA, Axin2, and MYOCD levels and/or activity are in aorta. In a preferred embodiment, the phosphosmad2, phosphosmad3, urinary protein, ActRIIA, and col1a1 levels and/or activity are in kidney. In a preferred embodiment, the activin levels and/or activity are in serum. In certain embodiments, the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, and/or Sm22-alpha are the protein level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix,

Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, and/or Sm22-alpha, respectively. In certain embodiments, the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, and/or Sm22-alpha are the mRNA level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, and/or Sm22-alpha, respectively. In certain embodiments, the levels of activin are elevated in the subject with respect to a reference population. In certain embodiments, the levels of follistatin in the subject are about equal to the levels of follistatin in a reference population.

[00134] In certain embodiments, the reference population is a population as described in Section 8.6. In certain embodiments, the subject is a subject as described in Section 8.4.

[00135] In certain embodiments, the ActRII signaling inhibitor is ActRIIA-Fc such as ActRIIA-hFc (*e.g.*, SEQ ID NO:7). In certain embodiments, the ActRII signaling inhibitor is an ActRII signaling inhibitor as described in Sections 8.5.

[00136] In certain embodiments, the pharmaceutically effective dose is a dose as described in Section 8.7. In certain embodiments, the pharmaceutically effective dose is an initial dose. In certain embodiments, the pharmaceutically effectively dose is administered at a frequency as described in Section 8.7. In certain embodiments, the pharmaceutically effective dose is administered as described in Section 8.7.

[00137] In certain embodiments, the methods provided herein are utilized in combination with a second pharmaceutically active agent, as described in Section 8.8.

[00138] In certain embodiments, provided herein are methods for treating end-stage kidney disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap), wherein the subject is on hemodialysis, and wherein the subject has previously been administered an erythropoietin-stimulating agent. In certain embodiments, provided herein are methods for treating end-stage kidney disease in a subject, comprising subcutaneously administering to the subject 0.13 mg/kg of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap) at an interval of once every 14 days, wherein the subject is on hemodialysis, and wherein the subject has previously been administered an erythropoietin-stimulating agent. In certain embodiments, provided herein are methods for treating end-stage kidney disease in a subject, comprising subcutaneously administering to the subject 0.26 mg/kg of an ActRII signaling inhibitor (*e.g.*, an activin ligand

trap) at an interval of once every 14 days, wherein the subject is on hemodialysis, and wherein the subject has previously been administered an erythropoietin-stimulating agent. In certain embodiments, provided herein are methods for treating end-stage kidney disease in a subject, comprising subcutaneously administering to the subject 0.3 mg/kg of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap) at an interval of once every 28 days, wherein the subject is on hemodialysis, and wherein the subject has previously been administered an erythropoietin-stimulating agent. In certain embodiments, provided herein are methods for treating end-stage kidney disease in a subject, comprising subcutaneously administering to the subject 0.5 mg/kg of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap) at an interval of once every 28 days, wherein the subject is on hemodialysis, and wherein the subject has previously been administered an erythropoietin-stimulating agent. In certain embodiments, provided herein are methods for treating end-stage kidney disease in a subject, comprising subcutaneously administering to the subject 0.7 mg/kg of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap) at an interval of once every 28 days, wherein the subject is on hemodialysis, and wherein the subject has previously been administered an erythropoietin-stimulating agent. In certain embodiments, provided herein are methods for treating end-stage kidney disease in a subject, comprising intravenously administering to the subject 0.1 mg/kg of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap) at an interval of once every 14 days, wherein the subject is on hemodialysis, and wherein the subject has previously been administered an erythropoietin-stimulating agent. In certain embodiments, provided herein are methods for treating end-stage kidney disease in a subject, comprising subcutaneously administering to the subject 0.2 mg/kg of an ActRII signaling inhibitor (*e.g.*, an activin ligand trap) at an interval of once every 14 days, wherein the subject is on hemodialysis, and wherein the subject has previously been administered an erythropoietin-stimulating agent. In certain embodiments, the ActRII signaling inhibitor is ActRIIA-hFc (SEQ ID NO:7).

8.3.4 ADJUSTED DOSING

[00139] In certain embodiments, the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha may be further used (i) to evaluate appropriate dosing for a subject, wherein the subject is a candidate to be treated or is being treated with an ActRII signaling inhibitor (*e.g.*, an activin ligand trap); (ii) to evaluate whether to adjust the dosage of the ActRII signaling inhibitor during treatment; and/or

(iii) to evaluate an appropriate maintenance dose of the ActRII signaling inhibitor. If the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha level and/or activity is greater than or less than the level and/or activity in a reference population, dosing with an ActRII signaling inhibitor may be initiated, increased, reduced, delayed or terminated depending on the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha, respectively. In certain embodiments, provided herein are methods for treating and/or preventing cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, cardiovascular disease associated with and/or resulting from renal disease, CKD-MBD, elevated levels of arterial stiffness, LVH, cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness, and/or cardiovascular disease associated with and/or resulting from LVH, in a subject comprising (i) administering to the subject an initial dose of an ActRII signaling inhibitor; (ii) taking a first measurement of the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; (iii) after a period of time, taking a second measurement of the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and (iv) administering to the subject an adjusted dose of the ActRII signaling inhibitor. In certain embodiments, the ActRII signaling inhibitor is ActRIIA-Fc such as ActRIIA-hFc (*e.g.*, SEQ ID NO:7). In certain embodiments, the ActRII signaling inhibitor is an ActRII signaling inhibitor as described in Section 8.5. In certain other embodiments, the subject has been diagnosed as having chronic kidney disease-mineral bone disorder. In certain other embodiments, the subject has not been diagnosed as having chronic kidney disease-mineral bone disorder. In certain embodiments, the vascular calcification in the subject is analyzed by measuring Agatston scores as described in Section 8.6.6. In certain embodiments, the adjusted dose is based on the detected change between the first measurement and the second measurement.

[00140] In certain embodiments, the levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA,

MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha are determined as described in Section 8.6. In certain embodiments, the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha are the protein level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, and/or Sm22-alpha, respectively. In certain embodiments, the level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha are the mRNA level and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or Sm22-alpha, respectively. In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or alpha-SMA levels are in a tissue. In certain embodiments, the tissue is aorta. In certain embodiments, the tissue is kidney. In certain embodiments, the tissue is bone. In certain embodiments, the tissue is serum. In a preferred embodiment, the Runx2, Dkk1, Alp, osterix, sm22-alpha, Klotho, alpha-SMA, MYOCD, ActRIIA, or Axin2 levels and/or activity are in aorta. In a preferred embodiment, the phosphosmad2, phosphosmad3, urinary protein, ActRIIA, Axin2, and col1a1 levels and/or activity are in kidney. In a preferred embodiment, the activin levels and/or activity are in serum. In certain embodiments, the levels of activin are elevated in the subject with respect to a reference population. In certain embodiments, the levels of follistatin are normal in the subject with respect to a reference population. In certain embodiments, the vascular calcification in the subject is analyzed by measuring Agatston scores as described in Section 8.6.

[00141] In certain embodiments, the reference population is a population as described in Section 8.6. In certain embodiments, the subject is a subject as described in Section 8.4.

[00142] In certain embodiments, the initial dose is a dose as described in Section 8.7. In certain embodiments, the initial dose is administered at a frequency as described in Section 8.7. In certain embodiments, the initial dose is administered as described in Section 8.7.

[00143] In certain embodiments, the first measurement and/or the second measurement is taken as described in Section 8.6. In certain embodiments, the first measurement is taken prior

to the commencement of the treatment. In certain embodiments, the first measurement is taken after the commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or two months thereof. In certain embodiments, the second measurement is taken immediately after commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months thereof.

[00144] In certain embodiments, the adjusted dose of the ActRII signaling inhibitor is greater than the initial dose if the level and/or activity of Runx2 is elevated as compared to the level and/or activity of Runx2 in a reference population, the level and/or activity of Alp is elevated as compared to the level and/or activity of Alp in a reference population, the level and/or activity of Snai1 is elevated as compared to the level and/or activity of Snai1 in a reference population, the level and/or activity of phosphosmad2 is elevated as compared to the level and/or activity of phosphosmad2 in a reference population, the level and/or activity of phosphosmad3 is elevated as compared to the level and/or activity of phosphosmad3 in a reference population, the level and/or activity of urinary protein is elevated as compared to the level and/or activity of urinary protein in a reference population, the level and/or activity of Dkk1 is elevated as compared to the level and/or activity of Dkk1 in a reference population, the level and/or activity of col1a1 is elevated as compared to the level and/or activity of col1a1 in a reference population, the level and/or activity of activin (*e.g.*, free activin) is elevated as compared to the level and/or activity of activin (*e.g.*, free activin) in a reference population, the level and/or activity of BSAP is elevated as compared to the level and/or activity of BSAP in a reference population, the level and/or activity of CTX is elevated as compared to the level and/or activity of CTX in a reference population, the level and/or activity of Osterix is elevated as compared to the level and/or activity of Osterix in a reference population, the level and/or activity of Klotho is decreased as compared to the level and/or activity of Klotho in a reference population, the level and/or activity of alpha-SMA is decreased as compared to the level and/or activity of alpha-SMA in a reference population, the level and/or activity of MYOCD is decreased as compared to the level and/or activity of MYOCD in a reference population, the level and/or activity of ActRIIA is decreased as compared to the level and/or activity of ActRIIA in a reference population, the level and/or activity of Axin2 is decreased as compared to the level and/or activity of Axin2 in a reference population, and/or the level and/or activity of Sm22-alpha

is decreased as compared to the level and/or activity of Sm22-alpha in a reference population. In certain embodiments, the elevated levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix are as described in Section 8.6. In certain embodiments, the decreased levels of Klotho, alpha-SMA, ALP, ActRIIA, Axin2, and/or Sm22-alpha are as described in Section 8.6. In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or alpha-SMA levels are in a tissue. In certain embodiments, the tissue is aorta. In certain embodiments, the tissue is kidney. In certain embodiments, the tissue is bone. In certain embodiments, the tissue is serum. In a preferred embodiment, the Runx2, Dkk1, Alp, osterix, sm22-alpha, Klotho, alpha-SMA, ActRIIA, Axin2, and MYOCD levels and/or activity are in aorta. In a preferred embodiment, the phosphosmad2, phosphosmad3, urinary protein, ActRIIA, Axin2, and col1a1 levels and/or activity are in kidney. In a preferred embodiment, the activin levels and/or activity are in serum.

[00145] In certain embodiments, the adjusted dose is a dose as described in Section 8.7. In certain embodiments, the adjusted dose is administered at a frequency as described in Section 8.7. In certain embodiments, the adjusted dose is administered as described in Section 8.7

[00146] In certain embodiments, the subject has elevated levels and/or activity of Runx2 as compared to prior levels and/or activity of Runx2 in the subject, elevated levels and/or activity of Alp as compared to prior levels and/or activity of Alp in the subject, elevated levels and/or activity of Snai1 as compared to prior levels and/or activity of Snai1 in the subject, elevated levels and/or activity of phosphosmad2 as compared to prior levels and/or activity of phosphosmad2 in the subject, elevated levels and/or activity of phosphosmad3 as compared to prior levels and/or activity of phosphosmad3 in the subject, elevated levels and/or activity of urinary protein as compared to prior levels and/or activity of urinary protein in the subject, elevated levels and/or activity of Dkk1 as compared to prior levels and/or activity of Dkk1 in the subject, elevated levels and/or activity of activin (*e.g.*, free activin) as compared to prior levels and/or activity of activin (*e.g.*, free activin) in the subject, elevated levels and/or activity of col1a1 as compared to prior levels and/or activity of col1a1 in the subject, elevated levels and/or activity of BSAP as compared to prior levels and/or activity of BSAP in the subject, elevated levels and/or activity of CTX as compared to prior levels and/or activity of CTX in the subject, elevated levels and/or activity of Osterix as compared to prior levels and/or activity of Osterix in

the subject, decreased levels and/or activity of Klotho as compared to prior levels and/or activity of Klotho in the subject, decreased levels and/or activity of alpha-SMA as compared to prior levels and/or activity of alpha-SMA in the subject, decreased levels and/or activity of MYOCD as compared to prior levels and/or activity of MYOCD in the subject, decreased levels and/or activity of ActRIIA as compared to prior levels and/or activity of ActRIIA in the subject, decreased levels and/or activity of Axin2 as compared to prior levels and/or activity of Axin2 in the subject, and/or decreased levels and/or activity of Sm22-alpha as compared to prior levels and/or activity of Sm22-alpha in the subject. In certain embodiments, the prior levels and/or activity of Runx2 in the subject, the prior levels and/or activity of Alp in the subject, the prior levels and/or activity of Snai1 in the subject, the prior levels and/or activity of phosphosmad2 in the subject, the prior levels and/or activity of phosphosmad3 in the subject, the prior levels and/or activity of urinary protein in the subject, the prior levels and/or activity of Dkk1 in the subject, the prior levels and/or activity of activin (*e.g.*, free activin) in the subject, the prior levels and/or activity of col1a1 in the subject, the prior levels and/or activity of BSAP in the subject, the prior levels and/or activity of CTX in the subject, the prior levels and/or activity of Osterix in the subject, the prior levels and/or activity of Klotho in the subject, the prior levels and/or activity of alpha-SMA in the subject, the prior levels and/or activity of MYOCD in the subject, the prior levels and/or activity of ActRIIA in the subject, the prior levels and/or activity of ActRIIA in the subject, the prior levels and/or activity of Axin2 in the subject, the prior levels and/or activity of Axin2 in the subject, and/or the prior levels and/or activity of Sm22-alpha in the subject are the respective levels 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, or 48 months before the onset of symptoms or the diagnosis of (i) cardiovascular disease; (ii) vascular calcification; (iii) cardiovascular disease associated with and/or resulting from vascular calcification; (iv) cardiovascular disease associated with and/or resulting from renal disease; (v) CKD-MBD; (vi) elevated levels of arterial stiffness; (vii) LVH; (viii) cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness; and/or (ix) cardiovascular disease associated with and/or resulting from LVH.

[00147] In certain embodiments, the adjusted dose of the ActRII signaling inhibitor (*e.g.*, an activin ligand trap) is less than the initial dose if the level and/or activity of Runx2 is decreased as compared to the level and/or activity of Runx2 in a reference population, the level and/or activity of Alp is decreased as compared to the level and/or activity of Alp in a reference population, the level and/or activity of Snai1 is decreased as compared to the level and/or activity

of Snai1 in a reference population, the level and/or activity of phosphosmad2 is decreased as compared to the level and/or activity of phosphosmad2 in a reference population, the level and/or activity of phosphosmad3 is decreased as compared to the level and/or activity of phosphosmad3 in a reference population, the level and/or activity of urinary protein is decreased as compared to the level and/or activity of urinary protein in a reference population, the level and/or activity of Dkk1 is decreased as compared to the level and/or activity of Dkk1 in a reference population, the level and/or activity of col1a1 is decreased as compared to the level and/or activity of col1a1 in a reference population, the level and/or activity of activin (*e.g.*, free activin) is decreased as compared to the level and/or activity of activin (*e.g.*, free activin) in a reference population, the level and/or activity of BSAP is decreased as compared to the level and/or activity of BSAP in a reference population, the level and/or activity of CTX is decreased as compared to the level and/or activity of CTX in a reference population, the level and/or activity of Osterix is decreased as compared to the level and/or activity of Osterix in a reference population, the level and/or activity of Klotho is elevated as compared to the level and/or activity of Klotho in a reference population, the level and/or activity of alpha-SMA is elevated as compared to the level and/or activity of alpha-SMA in a reference population, the level and/or activity of MYOCD is elevated as compared to the level and/or activity of MYOCD in a reference population, the level and/or activity of ActRIIA is elevated as compared to the level and/or activity of ActRIIA in a reference population, the level and/or activity of Axin2 is elevated as compared to the level and/or activity of Axin2 in a reference population, and/or the level and/or activity of Sm22-alpha is elevated as compared to the level and/or activity of Sm22-alpha in a reference population. In certain embodiments, the elevated levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are as described in Section 8.6. In certain embodiments, the decreased levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix are as described in Section 8.6. In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, phosphosmad3, urinary protein, ActRIIA, Axin2, and/or alpha-SMA levels are in a tissue. In certain embodiments, the tissue is aorta. In certain embodiments, the tissue is kidney. In certain embodiments, the tissue is bone. In certain embodiments, the tissue is serum. In a preferred embodiment, the Runx2, Dkk1, Alp, osterix, sm22-alpha, Klotho, alpha-SMA, ActRIIA, Axin2, and MYOCD levels and/or activity are in aorta. In a preferred embodiment, the phosphosmad2, phosphosmad3, urinary protein, ActRIIA,

Axin2, and col1a1 levels and/or activity are in kidney. In a preferred embodiment, the activin levels and/or activity are in serum. In certain embodiments, the Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, Sm22-alpha, MYOCD, ActRIIA, Axin2, and/or alpha-SMA levels are in aorta.

[00148] In certain embodiments, the reference population is a population as described in Section 8.6. In certain embodiments, the adjusted dose is a dose as described in Section 8.7. In certain embodiments, the adjusted dose is administered at a frequency as described in Section 8.7. In certain embodiments, the adjusted dose is administered as described in Section 8.7

[00149] In certain embodiments, the methods provided herein are utilized in combination with a second pharmaceutically active agent, as described in Section 8.8.

[00150] As will be recognized by one of skill in the art, levels and/or activity of one or more of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, Sm22-alpha, phosphosmad3, and urinary protein can be compared independently and respectively to the level and/or activity in a corresponding reference population, such as described above in Section 8.3.1.

8.4 PATIENT POPULATIONS

[00151] The subjects treated in accordance with the methods described herein can be any mammals such as rodents and primates, and in a preferred embodiment, humans. In certain embodiments, the methods described herein can be used to treat cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, cardiovascular disease associated with and/or resulting from renal disease, high-turnover ROD, elevated levels of arterial stiffness, cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness, LVH, and/or cardiovascular disease associated with and/or resulting from LVH; or to monitor and/or decrease vascular calcification, arterial (*e.g.*, vascular) stiffness, LVH, aortic calcium levels, endothelial to mesenchymal transition, and/or bone formation, and/or to increase bone mineral density and/or vascular smooth muscle cell function, in any mammals such as rodents and primates, and in a preferred embodiment, in human subjects.

[00152] In certain embodiments, the subject treated in accordance with the methods described herein has elevated levels of activin as compared to the levels of activin in a reference population. In certain embodiments, the activin levels are the kidney, plasma, or aortic activin levels. In certain embodiments, the activin is activin A. In certain embodiments, the subject

treated in accordance with the methods described herein has about equal levels of follistatin as compared to the levels of follistatin in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein does not have elevated levels of follistatin as compared to the levels of follistatin as compared to the levels of follistatin in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein does not have decreased levels of follistatin as compared to the levels of follistatin as compared to the levels of follistatin in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has about equal levels of follistatin-like 3 as compared to the levels of follistatin-like 3 in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein does not have elevated levels of follistatin-like 3 as compared to the levels of follistatin-like 3 as compared to the levels of follistatin-like 3 in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein does not have decreased levels of follistatin-like 3 as compared to the levels of follistatin-like 3 as compared to the levels of follistatin-like 3 in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has about equal levels of inhibin as compared to the levels of inhibin in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein does not have elevated levels of inhibin as compared to the levels of inhibin as compared to the levels of inhibin in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein does not have decreased levels of inhibin as compared to the levels of inhibin as compared to the levels of inhibin in a reference population. In certain embodiments, the level of free activin in a subject is determined by the stoichiometry of inhibin, follistatin, and follistatin-like 3 levels to activin levels. Accordingly, in certain embodiments, the subject treated in accordance with the methods described herein has an increased level of free activin.

[00153] In certain embodiments, the subject treated in accordance with the methods described herein does not have an increase in phosphoerk 1/2 as compared to a reference population as described in Section 8.6. In certain embodiments, the phosphoerk 1/2 is aortic phosphoerk 1/2.

[00154] In certain embodiments, the subject treated in accordance with the methods described herein has increased endothelial to mesenchymal transition (EnMT) as compared to EnMT in a reference population. In certain embodiments, the subject treated in accordance with

the methods described herein has increased expression of transcription factors associated with EnMT, such as, for example, Snai1. In certain embodiments, the increased EnMT in the subject results in increased osteoblastic transition in the subject as compared to the osteoblastic transition in a reference population. In certain embodiments, the increased EnMT in the subject results in increased vascular stiffness in the subject as compared to the vascular stiffness in a reference population. In certain embodiments, the increased EnMT in the subject results in increased vascular calcification in the subject as compared to the vascular calcification in a reference population. In certain embodiments, the increased EnMT in the subject results in decreased vascular smooth muscle function in the subject as compared to the vascular smooth muscle function in a reference population. In certain embodiments, the increased EnMT in the subject results in increased LVH in the subject as compared to the LVH in a reference population.

[00155] In certain embodiments, the subject treated in accordance with the methods provided herein has renal fibrosis. In certain embodiments, the subject treated in accordance with the methods provided herein has glomerulosclerosis.

[00156] In certain embodiments, the subject treated in accordance with the methods described herein has elevated levels and/or activity of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, phosphosmad3, urinary protein, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) vascular calcification; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) cardiovascular disease; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin),

Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) cardiovascular disease associated with and/or resulting from vascular calcification; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) cardiovascular disease associated with and/or resulting from renal disease; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) CKD-MBD; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) elevated levels of arterial stiffness; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the

levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) LVH; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the subject treated in accordance with the methods described herein has (i) cardiovascular disease associated with and/or resulting from LVH; and (ii) elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in a reference population.

[00157] In certain embodiments, the subject treated in accordance with the methods described herein has elevated levels and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, and/or decreased levels and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, as compared to prior levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho,

alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in the subject 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, or 48 months before the onset of symptoms or the diagnosis of (i) cardiovascular disease; (ii) vascular calcification; (iii) cardiovascular disease associated with and/or resulting from vascular calcification; (iv) cardiovascular disease associated with and/or resulting from renal disease; (v) CKD-MBD; (vi) elevated levels of arterial stiffness; (vii) LVH; (viii) cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness; and/or (ix) cardiovascular disease associated with and/or resulting from LVH.

[00158] In certain other embodiments, the subject has been diagnosed as having chronic kidney disease-mineral bone disorder. In certain other embodiments, the subject has not been diagnosed as having chronic kidney disease-mineral bone disorder. In certain embodiments, the reference population is a population as described in Section 8.6. In certain embodiments, the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix are as described in Section 8.6. In certain embodiments, the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha are as described in Section 8.6.

[00159] In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated Snai1 levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated phosphosmad2 levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated phosphosmad3 levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated urinary protein levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated Dkk1 levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated Col1a1 levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated Runx2 levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated Alp levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated BSAP levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated CTX levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease

associated with elevated Osterix levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with elevated ActRIIA levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with decreased Klotho levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with decreased alpha-SMA levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with decreased MYOCD levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with decreased Sm22-alpha levels. In certain embodiments, the subject treated in accordance with the methods provided herein has a disease associated with decreased ActRIIA levels.

[00160] In certain embodiments, the subject treated in accordance with the methods provided herein has cardiovascular disease. In certain embodiments, the subject treated in accordance with the methods provided herein has vascular calcification. In certain embodiments, the subject treated in accordance with the methods provided herein has a cardiovascular disease associated with and/or resulting from vascular calcification. In certain embodiments, the vascular calcification is determined as described in Section 8.6. In certain embodiments, the vascular calcification is determined by Agatston score. In certain embodiments, the vascular calcification is calcific atherosclerosis, calcific medial vasculopathy (also known as Mönckeberg's medial calcific sclerosis), medial calcification, elastocalcinosis, calcific uremic arterolopathy, calcific aortic valvular stenosis, and/or portal vein calcification. In certain embodiments, the disease associated with calcific atherosclerosis is atherosclerosis, hyperlipidemia, osteoporosis, hypertension, inflammation, type 2 diabetes mellitus, end-stage renal disease, required amputation, pseudoxanthoma elasticum, end-stage renal disease, hyperlipidemia, congenital bicuspid valve, rheumatic heart disease, and/or liver disease. In certain embodiments, the subject treated in accordance with the methods provided herein has CKD-induced increased heart weight. In certain embodiments, the subject has cardiac hypertrophy. In certain embodiments, the subject has myocyte hypertrophy. In certain embodiments, the subject has increased arterial and/or vascular stiffness. In certain embodiments, the subject has LVH.

[00161] In certain embodiments, the subject treated in accordance with the methods described herein has an undesirably high level of aortic calcium, vascular calcification, elevated

levels of arterial stiffness, and/or osteoblast numbers as compared to a reference population as described in Section 8.6. In certain embodiments, the subject treated in accordance with the methods described herein is at risk for developing undesirably high level of aortic calcium, vascular calcification, elevated levels of arterial stiffness, and/or osteoblast numbers, such as a subject with chronic kidney disease as compared to a reference population as described in Section 8.6. In certain embodiments, the subject treated in accordance with the methods provided herein has calcium deposits in aortic atheromas as compared to a reference population as described in Section 8.6. In certain embodiments, the subject treated in accordance with the methods provided herein has elevated levels of arterial stiffness in the form of aortic stiffness as compared to a reference population as described in Section 8.6. In certain embodiments, the subject treated in accordance with the methods provided herein has a level of elevated levels of arterial stiffness that increases hypertension as compared to a reference population as described in Section 8.6.

[00162] In certain embodiments, the subject treated in accordance with the methods provided herein has cardiovascular disease is associated with and/or results from renal disease. In certain embodiments, the renal disease is chronic kidney disease. In certain embodiments, the subject treated in accordance with the methods provided herein has chronic kidney disease. In certain embodiments, the subject has cardiovascular disease secondary to chronic kidney disease. In certain embodiments, the chronic kidney disease has reached stage 3, stage 4, stage 5, or stage 5D. In a specific embodiment, the kidney disease is end-stage kidney disease. In certain embodiments, the subject has a glomerular filtration rate of less than 60 ml/min/1.73m^2 in adults or less than 89 ml/min/1.73m^2 in pediatric subjects. *See*, Moe *et al.*, 2006, Kidney International 69:1945-1953. In certain embodiments, the subject is an adult, wherein the subject has a glomerular filtration rate of less than 50 ml/min/1.73m^2, 40 ml/min/1.73m^2, 30 ml/min/1.73m^2, 20 ml/min/1.73m^2, or less than 10 ml/min/1.73m^2. In certain embodiments, the subject is a pediatric subject, wherein the pediatric subject has a glomerular filtration rate of less than 80 ml/min/1.73m^2, 70 ml/min/1.73m^2, 60 ml/min/1.73m^2, 50 ml/min/1.73m^2, 40 ml/min/1.73m^2, 30 ml/min/1.73m^2, 20 ml/min/1.73m^2, or less than 10 ml/min/1.73m^2. Without being bound by theory, a glomerular filtration rate of less than 60 ml/min/1.73m^2 in adult subjects and less than 89 ml/min/1.73m^2 in pediatric subjects results in detectable abnormalities in calcium levels, phosphorus levels, PTH levels, and vitamin D metabolism; and abnormal levels of these markers result in bone disease. In certain embodiments, the subject has reduced inulin clearance a

reference population, such as a reference population as described in Section 8.6. In certain embodiments, the subject has reduced blood urea nitrogen levels a reference population, such as a reference population as described in Section 8.6. In certain embodiments, the subject is hyperphosphatemic. In certain embodiments, the subject has renal fibrosis.

[00163] In certain embodiments, the subject has Alport's syndrome. In certain embodiments, the subject has one or more somatic mutations in the COL4A5 gene associated with Alport's syndrome.

[00164] In certain embodiments, the subject treated in accordance with the methods provided herein has a bone pathology associated with chronic kidney disease, *i.e.*, CKD-Mineral/Bone Disorder (CKD-MBD). *See* Moe *et al.*, 2006, Kidney International 69:1945-1953. In certain embodiments, the subject has decreased trabecular bone volume as compared to trabecular bone volume in a reference population, such as a reference population as described in Section 8.6. In certain embodiments, the subject has decreased trabecular thickness as compared to trabecular thickness in a reference population, such as a reference population as described in Section 8.6. In certain embodiments, the subject has increased erosion surface to bone surface ratio as compared to erosion surface to bone surface ratio in a reference population, such as a reference population as described in Section 8.6. In certain embodiments, the subject has increased osteoclast levels as compared to osteoclast levels in a reference population, such as a reference population as described in Section 8.6. In certain embodiments, the subject has increased osteoblast surface to bone surface ratio as compared to osteoblast surface to bone surface ratio in a reference population, such as a reference population as described in Section 8.6. In certain embodiments, the subject has increased osteoblast levels as compared to osteoblast levels in a reference population, such as a reference population as described in Section 8.6. In certain embodiments, the subject has reduced bone formation rate as compared to bone formation rate in a reference population, such as a reference population as described in Section 8.6. In certain embodiments, the CKD-MBD consists of renal osteodystrophy and vascular calcification. In certain embodiments, the CKD-MBD consists of renal osteodystrophy. In certain embodiments, the CKD-MBD is low-turnover CKD-MBD. In certain embodiments, the CKD-MBD consists of elevated levels of arterial stiffness and/or LVH. Low-turnover CKD-MBD can be diagnosed by the histological features set forth in Table 1 below. *See* National Kidney Foundation, Kidney Disease Outcomes Quality Initiative Guidelines at the website of the National Kidney Foundation. In certain embodiments, the subject has high-turnover ROD.

[00165] **Table 1.** Histological Features of Low-Turnover CKD-MBD

Feature	Adynamic	Osteomalacia
Bone Formation		
Trabecular bone volume	Normal, low	Variable Low, normal or high
Osteoid volume	Normal, low	High-very high
Osteoid seam thickness	Normal, low	High-very high
Number of osteoblasts	Low	Low
Bone formation rate	Low-very low	Low-very low
Mineralization lag time	Normal	Prolonged
Bone Resorption		
Eroded bone perimeter	Normal, low	Variable Often low, may be high
Number of osteoclasts	Low	Low, may be normal or high
Marrow fibrosis	Absent	Absent

[00166] In certain embodiments, the subject is undergoing hemodialysis.

[00167] In certain embodiments, the subject treated in accordance with the methods described herein has end-stage renal disease. In certain embodiments, the subject treated in accordance with the methods described herein undergoes dialysis.

[00168] In certain embodiments, the subject treated in accordance with the methods described herein has increased bone resorption as compared to bone resorption in a reference population. In certain embodiments, the increased bone resorption is determined by the levels of CTX. In certain embodiments, the levels of CTX are determined as described in Section 8.6. In certain embodiments, the bone resorption is assessed as described in Section 8.6.

[00169] In certain embodiments, the subject treated in accordance with the methods described here can be of any age. In certain embodiments, the subject treated in accordance with the methods described herein is less than 18 years old. In a specific embodiment, the subject treated in accordance with the methods described herein is less than 13 years old. In another specific embodiment, the subject treated in accordance with the methods described herein is less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, or less than 5 years old. In another specific embodiment, the subject treated in accordance with the methods described herein is 1-3 years old, 3-5 years old, 5-7 years old, 7-9 years old, 9-11 years old, 11-13 years old, 13-15 years old, 15-20 years old, 20-25 years old, 25-30 years old, or greater than 30 years old. In another specific embodiment, the subject treated in accordance with the methods described herein is 30-35 years old, 35-40 years old, 40-45 years old, 45-50 years old, 50-55 years old, 55-60 years old, or greater than 60 years old. In another specific embodiment,

the subject treated in accordance with the methods described herein is 60-65 years old, 65-70 years old, 70-75 years old, 75-80 years old, or greater than 80 years old.

[00170] In certain embodiments, a subject treated in accordance with the methods provided herein has end-stage kidney disease. In certain embodiments, a subject treated in accordance with the methods provided herein is on hemodialysis. In certain embodiments, a subject treated in accordance with the methods provided herein has previously been administered an erythropoietin-stimulating agent. In certain embodiments, a subject treated in accordance with the methods provided herein has end-stage kidney disease, is on hemodialysis, and has previously been administered an erythropoietin-stimulating agent.

[00171] As will be recognized by one of skill in the art, levels and/or activity of one or more of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, Sm22-alpha, phosphosmad3, and urinary protein can be compared independently and respectively to the level and/or activity in a corresponding reference population, such as described above in Section 8.3.1.

8.5 INHIBITORS OF ACTRII SIGNALING

[00172] The ActRII signaling inhibitors described in this section and known in the art can be used in the methods provided herein. In certain embodiments, the ActRII signaling inhibitors described in this section can be used in the methods provided herein (*See*, Section 8.3).

[00173] Inhibitors of ActRII signaling receptors encompassed herein include ActRIIA signaling inhibitors and ActRIIB signaling inhibitors (*see* below). In certain embodiments, an ActRII signaling inhibitor is specific to ActRIIA signaling. In other embodiments, an ActRII signaling inhibitor is specific to ActRIIB signaling. In certain embodiments, an ActRII signaling inhibitor preferentially inhibits ActRIIA signaling. In other embodiments, an ActRII signaling inhibitor preferentially inhibits ActRIIB signaling. In certain embodiments, an ActRII signaling inhibitor inhibits both ActRIIA signaling and ActRIIB signaling.

[00174] In certain embodiments, inhibitors of ActRII signaling can be polypeptides comprising activin-binding domains of ActRII. Without being bound by theory, such activin-binding domain comprising polypeptides sequester activin and thereby prevent activin signaling. These activin-binding domain comprising polypeptides may comprise all or a portion of the extracellular domain of an ActRII (*i.e.*, all or a portion of the extracellular domain of ActRIIA or all or a portion of the extracellular domain of ActRIIB). In specific embodiments, the extracellular domain of an ActRII is soluble.

[00175] In certain embodiments, the activin-binding domain comprising polypeptides are linked to an Fc portion of an antibody (*e.g.*, a conjugate comprising an activin-binding domain comprising polypeptide of an ActRII receptor and an Fc portion of an antibody is generated). Without being bound by theory, the antibody portion confers increased stability on the conjugate. In certain embodiments, the activin-binding domain is linked to an Fc portion of an antibody via a linker, *e.g.*, a peptide linker.

[00176] The inhibitors of ActRII signaling used in the compositions and methods described herein comprise molecules that inhibit ActRIIA signaling and/or ActRIIB signaling, directly or indirectly, either extracellularly or intracellularly. In some embodiments, the inhibitors of ActRIIA signaling and/or ActRIIB signaling used in the compositions and methods described herein inhibit ActRIIA signaling and/or ActRIIB signaling via interactions with the receptor(s) itself. In other embodiments, the inhibitors of ActRIIA signaling and/or ActRIIB signaling used in the compositions and methods described herein inhibit ActRIIA signaling and/or ActRIIB signaling via interactions with an ActRIIA and/or ActRIIB ligand, *e.g.*, Activin.

8.5.1 INHIBITORS OF ACTRIIA SIGNALING

[00177] As used herein, the term "ActRIIA" refers to a family of activin receptor type IIA (ActRIIA) proteins from any species and variants derived from such ActRIIA proteins by mutagenesis or other modification. Reference to ActRIIA herein is understood to be a reference to any one of the currently identified forms. Members of the ActRIIA family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

[00178] ActRIIA signaling inhibitors to be used in the compositions and methods described herein include, without limitation, activin-binding soluble ActRIIA polypeptides; antibodies that bind to activin (particularly the activin A or B subunits, also referred to as β_A or β_B) and disrupt ActRIIA binding; antibodies that bind to ActRIIA and disrupt activin binding; non-antibody proteins selected for activin or ActRIIA binding (*see e.g.*, WO/2002/088171, WO/2006/055689, WO/2002/032925, WO/2005/037989, US 2003/0133939, and US 2005/0238646, each of which is incorporated herein by reference in its entirety, for examples of such proteins and methods for design and selection of same); and randomized peptides selected for activin or ActRIIA binding, which can be conjugated to an Fc domain.

[00179] In certain embodiments, two or more different proteins (or other moieties) with activin or ActRIIA binding activity, especially activin binders that block the type I (*e.g.*, a soluble type I activin receptor) and type II (*e.g.*, a soluble type II activin receptor) binding sites, respectively, may be linked together to create a bifunctional or multifunctional binding molecule that inhibits ActRIIA signaling and thus can be used in the compositions and methods described herein. In certain embodiments, Activin-ActRIIA signaling axis antagonists that inhibit ActRIIA signaling include nucleic acid aptamers, small molecules and other agents are used in the compositions and methods described herein include.

8.5.1.1 ActRIIA Signaling Inhibitors Comprising ActRIIA Polypeptides

[00180] The term "ActRIIA polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ActRIIA family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. For example, ActRIIA polypeptides include polypeptides derived from the sequence of any known ActRIIA having a sequence at least about 80% identical to the sequence of an ActRIIA polypeptide, and optionally at least 85%, 90%, 95%, 97%, 98%, 99% or greater identity. For example, an ActRIIA polypeptide may bind to and inhibit the function of an ActRIIA protein and/or activin. An ActRIIB polypeptide may be selected for its ability to promote bone growth and bone mineralization. Examples of ActRIIA polypeptides include human ActRIIA precursor polypeptide (SEQ ID NO: 1) and soluble human ActRIIA polypeptides (*e.g.*, SEQ ID NOS: 2, 3, 7 and 12). With respect to the ActRIIA precursor polypeptide whose amino acid sequence is depicted at SEQ ID NO:1, the signal peptide of the human ActRIIA precursor polypeptide located at amino acid positions 1 to 20; the extracellular domain is located at amino acid positions 21 to 135 and the N-linked glycosylation sites of the human ActRIIA precursor polypeptide (SEQ ID NO: 1) are located at amino acid positions 43 and 56 of SEQ ID NO:1. The nucleic acid sequence encoding the human ActRIIB precursor polypeptide of SEQ ID NO:1 is disclosed as SEQ ID NO:4 (nucleotides 164-1705 of Genbank entry NM_001616). The nucleic acid sequence encoding the soluble human ActRIIA polypeptide of SEQ ID NO:2 is disclosed as SEQ ID NO:5. *See* Table 21 for a description of the sequences.

[00181] In specific embodiments, the ActRIIA polypeptides used in the compositions and methods described herein are soluble ActRIIA polypeptides. An extracellular domain of an ActRIIA protein can bind to activin and is generally soluble, and thus can be termed a soluble, activin-binding ActRIIA polypeptide. Thus, as used herein, the term "soluble ActRIIA

polypeptide" generally refers to polypeptides comprising an extracellular domain of an ActRIIA protein, including any naturally occurring extracellular domain of an ActRIIA protein as well as any variants thereof (including mutants, fragments and peptidomimetic forms). Soluble ActRIIA polypeptides can bind to activin; however, the wild type ActRIIA protein does not exhibit significant selectivity in binding to activin versus GDF8/11. Native or altered ActRIIA proteins may be given added specificity for activin by coupling them with a second, activin-selective binding agent. Examples of soluble, activin-binding ActRIIA polypeptides include the soluble polypeptides illustrated in SEQ ID NOS: 2, 3, 7, 12 and 13. Other examples of soluble, activin-binding ActRIIA polypeptides comprise a signal sequence in addition to the extracellular domain of an ActRIIA protein, for example, the honey bee mellitin leader sequence (SEQ ID NO: 8), the tissue plasminogen activator (TPA) leader (SEQ ID NO: 9) or the native ActRIIA leader (SEQ ID NO: 10). The ActRIIA-hFc polypeptide illustrated in SEQ ID NO:13 uses a TPA leader.

[00182] In certain embodiments, the inhibitors of ActRIIA signaling used in the compositions and methods described herein comprise a conjugate/fusion protein comprising an activin-binding domain of ActRIIA linked to an Fc portion of an antibody. In certain embodiments, the activin-binding domain is linked to an Fc portion of an antibody via a linker, *e.g.*, a peptide linker. Optionally, the Fc domain has one or more mutations at residues such as Asp-265, lysine 322, and Asn-434. In certain cases, the mutant Fc domain having one or more of these mutations (*e.g.*, an Asp-265 mutation) has a reduced ability to bind to the Fcγ receptor relative to a wild-type Fc domain. In other cases, the mutant Fc domain having one or more of these mutations (*e.g.*, an Asn-434 mutation) has an increased ability to bind to the MHC class I-related Fc-receptor (FcRN) relative to a wild-type Fc domain. Exemplary fusion proteins comprising a soluble extracellular domain of ActRIIA fused to an Fc domain are set forth in SEQ ID NOs: 6, 7, 12, and 13.

[00183] In a specific embodiment, the ActRIIA signaling inhibitors used in the compositions and methods described herein comprise the extracellular domain of ActRIIA, or a portion thereof, linked to an Fc portion of an antibody, wherein said ActRIIA signaling inhibitor comprises an amino acid sequence that is at least 75% identical to an amino acid sequence selected from SEQ ID NOs: 6, 7, 12, and 13. In another specific embodiment, the ActRIIA signaling inhibitors used in the compositions and methods described herein comprise the extracellular domain of ActRIIA, or a portion thereof, linked to an Fc portion of an antibody, wherein said ActRIIA signaling inhibitor comprises an amino acid sequence that is at least 80%,

85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOs: 6, 7, 12, and 13.

[00184] In certain embodiments, the inhibitors of ActRIIA signaling used in the compositions and methods described herein comprise a truncated form of an extracellular domain of ActRIIA. The truncation can be at the carboxy terminus and/or the amino terminus of the ActRIIA polypeptide. In certain embodiments, the truncation can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids long relative to the mature ActRIIB polypeptide extracellular domain. In certain embodiments, the truncation can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 N-terminal amino acids of the mature ActRIIA polypeptide extracellular domain. In certain embodiments, the truncation can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 C-terminal amino acids of the mature ActRIIA polypeptide extracellular domain. For example, truncated forms of ActRIIA include polypeptides with amino acids 20-119; 20-128; 20-129; 20-130; 20-131; 20-132; 20-133; 20-134; 20-131; 21-131; 22-131; 23-131; 24-131; and 25-131, wherein the amino acid positions refer to the amino acid positions in SEQ ID NO:1.

[00185] In certain embodiments, the inhibitors of ActRIIA signaling used in the compositions and methods described herein comprise an extracellular domain of ActRIIA with one or more amino acid substitutions. In certain embodiments, the inhibitors of ActRIIA signaling used in the compositions and methods described herein comprise a truncated form of an ActRIIA extracellular domain that also carries an amino acid substitution.

[00186] In a specific embodiment, the ActRIIA signaling inhibitor to be used in the compositions and methods described herein is a fusion protein between the extracellular domain of the human ActRIIA receptor and the Fc portion of IgG1. In another specific embodiment, the ActRIIA signaling inhibitor to be used in the compositions and methods described herein is a fusion protein between a truncated extracellular domain of the human ActRIIA receptor and the Fc portion of IgG1. In another specific embodiment, the ActRIIA signaling inhibitor to be used in the compositions and methods described herein is a fusion protein between a truncated extracellular domain of the human ActRIIA receptor and the Fc portion of IgG1, wherein the truncated extracellular domain of the human ActRIIA receptor possesses one or more amino acid substitutions.

[00187] Functionally active fragments of ActRIIA polypeptides can be obtained, for example, by screening polypeptides recombinantly produced from the corresponding fragment of

the nucleic acid encoding an ActRIIA polypeptide. In addition, fragments can be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those peptidyl fragments that can function as antagonists (inhibitors) of ActRIIA protein or signaling mediated by activin.

[00188] In addition, functionally active variants of ActRIIA polypeptides can be obtained, for example, by screening libraries of modified polypeptides recombinantly produced from the corresponding mutagenized nucleic acids encoding an ActRIIA polypeptide. The variants can be produced and tested to identify those that can function as antagonists (inhibitors) of ActRIIA protein or signaling mediated by activin. In certain embodiments, a functional variant of the ActRIIA polypeptides comprises an amino acid sequence that is at least 75% identical to an amino acid sequence selected from SEQ ID NOs: 2 or 3. In certain cases, the functional variant has an amino acid sequence at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 2 or 3.

[00189] Functional variants may be generated, for example, by modifying the structure of an ActRIIA polypeptide for such purposes as enhancing therapeutic efficacy, or stability (*e.g.*, ex vivo shelf life and resistance to proteolytic degradation in vivo). Such modified ActRIIA polypeptides when selected to retain activin binding, can be considered functional equivalents of the naturally-occurring ActRIIA polypeptides. Modified ActRIIA polypeptides can also be produced, for instance, by amino acid substitution, deletion, or addition. For instance, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*e.g.*, conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of an ActRIIA polypeptide results in a functional homolog can be readily determined by assessing the ability of the variant ActRIIA polypeptide to produce a response in cells in a fashion similar to the wild-type ActRIIA polypeptide.

[00190] In certain embodiments, the ActRIIA signaling inhibitor to be used in the compositions and methods described herein provided herein may comprise an ActRIIA polypeptide having one or more specific mutations that can alter the glycosylation of the polypeptide. Such mutations may introduce or eliminate one or more glycosylation sites, such as

O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine (or asparagines-X-serine) (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the wild-type ActRIIA polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on an ActRIIA polypeptide is by chemical or enzymatic coupling of glycosides to the ActRIIA polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston (1981) CRC Crit. Rev. Biochem., pp. 259-306, incorporated by reference herein. Removal of one or more carbohydrate moieties present on an ActRIIA polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of the ActRIIA polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Chemical deglycosylation is further described by Hakimuddin *et al.* (1987) Arch. Biochem. Biophys. 259:52 and by Edge *et al.* (1981) Anal. Biochem. 118:131. Enzymatic cleavage of carbohydrate moieties on ActRIIA polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* (1987) Meth. Enzymol. 138:350. The sequence of an ActRIIA polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, ActRIIA proteins for use in humans can be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other expression systems, such as other mammalian expression cell lines, yeast cell lines with engineered glycosylation enzymes and insect cells, are expected to be useful as well.

[00191] Further provided herein are methods of generating mutants, particularly sets of combinatorial mutants of an ActRIIA polypeptide, as well as truncation mutants; pools of combinatorial mutants are especially useful for identifying functional variant sequences. The purpose of screening such combinatorial libraries may be to generate, for example, ActRIIA polypeptide variants which can act as either agonists or antagonist, or alternatively, which possess novel activities all together. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, an ActRIIA polypeptide variant may be screened for ability to bind to an ActRIIA ligand, to prevent binding of an ActRIIA ligand to an ActRIIA polypeptide or to interfere with signaling caused by an ActRIIA ligand.

[00192] Combinatorially-derived variants can be generated which have a selective or generally increased potency relative to a naturally occurring ActRIIA polypeptide. Likewise, mutagenesis can give rise to variants which have intracellular half-lives dramatically different than the corresponding a wild-type ActRIIA polypeptide. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other cellular processes which result in destruction of, or otherwise inactivation of a native ActRIIA polypeptide. Such variants, and the genes which encode them, can be utilized to alter ActRIIA polypeptide levels by modulating the half-life of the ActRIIA polypeptides. For instance, a short half-life can give rise to more transient biological effects and can allow tighter control of recombinant ActRIIA polypeptide levels within the subject. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter the half-life of the protein.

[00193] A combinatorial library may be produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential ActRIIA polypeptide sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential ActRIIA polypeptide nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display).

[00194] There are many ways by which the library of potential homologs can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art (*see*, for example, Narang, S A (1983) Tetrahedron 39:3; Itakura *et al.*, (1981) Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam:

Elsevier pp 273-289; Itakura *et al.*, (1984) Annu. Rev. Biochem. 53:323; Itakura *et al.*, (1984) Science 198:1056; Ike *et al.*, (1983) Nucleic Acid Res. 11:477). Such techniques have been employed in the directed evolution of other proteins (*see*, for example, Scott *et al.*, (1990) Science 249:386-390; Roberts *et al.*, (1992) PNAS USA 89:2429-2433; Devlin *et al.*, (1990) Science 249: 404-406; Cwirla *et al.*, (1990) PNAS USA 87: 6378-6382; as well as U.S. Pat. Nos. 5,223,409, 5,198,346, and 5,096,815).

[00195] Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, ActRIIA polypeptide variants can be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis and the like (Ruf *et al.*, (1994) Biochemistry 33:1565-1572; Wang *et al.*, (1994) J. Biol. Chem. 269:3095-3099; Balint *et al.*, (1993) Gene 137:109-118; Grodberg *et al.*, (1993) Eur. J. Biochem. 218:597-601; Nagashima *et al.*, (1993) J. Biol. Chem. 268:2888-2892; Lowman *et al.*, (1991) Biochemistry 30:10832-10838; and Cunningham *et al.*, (1989) Science 244:1081-1085), by linker scanning mutagenesis (Gustin *et al.*, (1993) Virology 193:653-660; Brown *et al.*, (1992) Mol. Cell Biol. 12:2644-2652; McKnight *et al.*, (1982) Science 232:316); by saturation mutagenesis (Meyers *et al.*, (1986) Science 232:613); by PCR mutagenesis (Leung *et al.*, (1989) Method Cell Mol Biol 1:11-19); or by random mutagenesis, including chemical mutagenesis, etc. (Miller *et al.*, (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, N.Y.; and Greener *et al.*, (1994) Strategies in Mol Biol 7:32-34). Linker scanning mutagenesis, particularly in a combinatorial setting, is an attractive method for identifying truncated (bioactive) forms of ActRIIA polypeptides.

[00196] A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and, for that matter, for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of ActRIIA polypeptides. The most widely used techniques for screening large gene libraries typically comprises cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include activin binding assays and activin-mediated cell signaling assays.

[00197] In certain embodiments, ActRIIA polypeptides used in the inhibitors of the methods and compositions described herein may further comprise post-translational modifications in addition to any that are naturally present in the ActRIIA polypeptides. Such modifications may include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, the modified ActRIIA polypeptides may contain non-amino acid elements, such as polyethylene glycols, lipids, poly- or mono-saccharide, and phosphates. Effects of such non-amino acid elements on the functionality of an ActRIIA polypeptide may be tested by any method known to the skilled artisan. When an ActRIIA polypeptide is produced in cells by cleaving a nascent form of the ActRIIA polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (such as CHO, HeLa, MDCK, 293, W138, NIH-3T3 or HEK293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the ActRIIA polypeptides.

[00198] In certain aspects, functional variants or modified forms of the ActRIIA polypeptides used in the inhibitors of the methods and compositions described herein include fusion proteins having at least a portion of the ActRIIA polypeptides and one or more fusion domains. Well known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt-conjugated resins are used. Many of such matrices are available in "kit" form, such as the Pharmacia GST purification system and the QIAexpress.TM. system (Qiagen) useful with (HIS_6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ActRIIA polypeptides. Examples of such detection domains include the various fluorescent proteins (*e.g.*, GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus hemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for Factor Xa or Thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the

recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation. In certain preferred embodiments, an ActRIIA polypeptide is fused with a domain that stabilizes the ActRIIA polypeptide in vivo (a "stabilizer" domain). By "stabilizing" is meant anything that increases serum half life, regardless of whether this is because of decreased destruction, decreased clearance by the kidney, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin are known to confer desirable pharmacokinetic properties on a wide range of proteins. Likewise, fusions to human serum albumin can confer desirable properties. Other types of fusion domains that may be selected include multimerizing (*e.g.*, dimerizing, tetramerizing) domains and functional domains (that confer an additional biological function, such as further stimulation of bone growth or muscle growth, as desired).

[00199] It is understood that different elements of the fusion proteins may be arranged in any manner that is consistent with the desired functionality. For example, an ActRIIA polypeptide may be placed C-terminal to a heterologous domain, or, alternatively, a heterologous domain may be placed C-terminal to an ActRIIA polypeptide. The ActRIIA polypeptide domain and the heterologous domain need not be adjacent in a fusion protein, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains.

[00200] In certain embodiments, the ActRIIA polypeptides used in the inhibitors of the methods and compositions described herein may contain one or more modifications that are capable of stabilizing the ActRIIA polypeptides. For example, such modifications may enhance the in vitro half life of the ActRIIA polypeptides, enhance circulatory half life of the ActRIIA polypeptides or reduce proteolytic degradation of the ActRIIA polypeptides. Such stabilizing modifications may include, but are not limited to, fusion proteins (including, for example, fusion proteins comprising an ActRIIA polypeptide and a stabilizer domain), modifications of a glycosylation site (including, for example, addition of a glycosylation site to an ActRIIA polypeptide), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from an ActRIIA polypeptide). In the case of fusion proteins, an ActRIIA polypeptide is fused to a stabilizer domain such as an IgG molecule (*e.g.*, an Fc domain). As used herein, the term "stabilizer domain" not only refers to a fusion domain (*e.g.*, Fc) as in the case of fusion proteins, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous polymer, such as polyethylene glycol.

[00201] In certain embodiments, isolated and/or purified forms of ActRIIA polypeptides, which are isolated from, or otherwise substantially free of, other proteins can be used with the methods and compositions described herein. ActRIIA polypeptides can generally be produced by expression from recombinant nucleic acids.

[00202] In certain aspects, the ActRIIA polypeptides used in the compositions and methods described herein are generated using isolated and/or recombinant nucleic acids encoding any of the ActRIIA polypeptides (*e.g.*, soluble ActRIIA polypeptides), including fragments, functional variants and fusion proteins disclosed herein. For example, SEQ ID NO: 4 encodes the naturally occurring human ActRIIA precursor polypeptide, while SEQ ID NO: 5 encodes the processed extracellular domain of ActRIIA. Such nucleic acids may be single-stranded or double stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making ActRIIA polypeptides or as direct therapeutic agents (*e.g.*, in a gene therapy approach).

[00203] In certain aspects, nucleic acids encoding ActRIIA polypeptides may include nucleic acids that are variants of SEQ ID NO: 4 or 5. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants.

[00204] In certain embodiments, isolated or recombinant nucleic acid sequences encoding ActRIIA polypeptides may be least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 4 or 5. One of ordinary skill in the art will appreciate that nucleic acid sequences complementary to SEQ ID NO: 4 or 5, and variants of SEQ ID NO: 4 or 5 may be used in the production of ActRIIA polypeptides suitable for use in the methods and compositions described herein. In further embodiments, such nucleic acid sequences can be isolated, recombinant, and/or fused to a heterologous nucleotide sequence, or be from a DNA library.

[00205] In other embodiments, nucleic acids used in the production of ActRIIA polypeptides suitable for use in the methods and compositions described herein may include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequence designated in SEQ ID NO: 4 or 5, complement sequence of SEQ ID NO: 4 or 5, or fragments thereof. One of ordinary skill in the art will understand that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one can perform the hybridization at 6.0 times sodium chloride/sodium citrate (SSC) at about 45 degree Celsius, followed by a wash of 2.0 times SSC at 50 degree Celsius. For example, the salt concentration

in the wash step can be selected from a low stringency of about 2.0 times SSC at 50 degree Celsius to a high stringency of about 0.2 times SSC at 50 degree Celsius. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22 degree Celsius, to high stringency conditions at about 65 degree Celsius. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In one embodiment, nucleic acids which hybridize under low stringency conditions of 6 times SSC at room temperature followed by a wash at 2 times SSC at room temperature can be used with the methods and compositions described herein.

[00206] Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NOs: 4 or 5 due to degeneracy in the genetic code also can be used in the production of ActRIIA polypeptides suitable for use in the methods and compositions described herein. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of a given species due to natural allelic variation.

[00207] In certain embodiments, the recombinant nucleic acids may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated herein. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome. In a preferred embodiment, the expression vector contains a selectable marker

gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

[00208] In certain aspects, the a nucleic acid used in the production of ActRIIA polypeptides suitable for use in the methods and compositions described herein can be provided in an expression vector comprising a nucleotide sequence encoding an ActRIIA polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the ActRIIA polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; Gene Expression Technology: Methods in Enzymology, Academic Press, San Diego, Calif. (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding an ActRIIA polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda, the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, *e.g.*, Pho5, the promoters of the yeast .alpha.-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

[00209] A recombinant nucleic acid used in the production of ActRIIA polypeptides suitable for use in the methods and compositions described herein can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression vehicles for production of a recombinant ActRIIA polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the types: pBR322-derived plasmids, pEMBL-

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

[00210] Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, *see* Molecular Cloning A Laboratory Manual, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the .beta.-gal containing pBlueBac III).

[00211] Vectors can be designed for production of the subject ActRIIA polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wis.). As will be apparent, the subject gene constructs can be used to cause expression of the subject ActRIIA polypeptides in cells propagated in culture, *e.g.*, to produce proteins, including fusion proteins or variant proteins, for purification.

[00212] Host cells transfected with a recombinant gene including a coding sequence (*e.g.*, SEQ ID NO: 4 or 5) for one or more of the subject ActRIIA polypeptides can be used in the production of ActRIIA polypeptides suitable for use in the methods and compositions described herein. The host cell may be any prokaryotic or eukaryotic cell. For example, an ActRIIA polypeptide provided herein may be expressed in bacterial cells such as E. coli, insect cells (*e.g.*,

using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art.

[00213] Accordingly, provided herein are methods of producing the ActRIIA polypeptides. For example, a host cell transfected with an expression vector encoding an ActRIIA polypeptide can be cultured under appropriate conditions to allow expression of the ActRIIA polypeptide to occur. The ActRIIA polypeptide may be secreted and isolated from a mixture of cells and medium containing the ActRIIA polypeptide. Alternatively, the ActRIIA polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art. The subject ActRIIA polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the ActRIIA polypeptides and affinity purification with an agent that binds to a domain fused to the ActRIIA polypeptide (*e.g.*, a protein A column may be used to purify an ActRIIA-Fc fusion). In a preferred embodiment, the ActRIIA polypeptide is a fusion protein containing a domain which facilitates its purification. In one embodiment, purification is achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. As demonstrated herein, ActRIIA-hFc protein was purified to a purity of >98% as determined by size exclusion chromatography and >95% as determined by SDS PAGE. This level of purity was sufficient to achieve desirable effects on bone in mice and an acceptable safety profile in mice, rats and non-human primates.

[00214] In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of a recombinant ActRIIA polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni^{2+} metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified ActRIIA polypeptide (*e.g.*, *see* Hochuli *et al.*, (1987) J. Chromatography 411:177; and Janknecht *et al.*, PNAS USA 88:8972).

[00215] Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence (*see*, for example, Current Protocols in Molecular Biology, eds. Ausubel *et al.*, John Wiley & Sons: 1992).

[00216] ActRIIA-Fc fusion protein can be expressed in stably transfected CHO-DUKX Bl 1 cells from a pAID4 vector (SV40 ori/enhancer, CMV promoter), using a tissue plasminogen leader sequence of SEQ ID NO:9. The Fc portion is a human IgGl Fc sequence, as shown in SEQ ID NO:7. In certain embodiments, upon expression, the protein contained has, on average, between about 1.5 and 2.5 moles of sialic acid per molecule of ActRIIA-Fc fusion protein.

[00217] In certain embodiments, the long serum half-life of an ActRIIA-Fc fusion can be 25-32 days in human subjects. Additionally, the CHO cell expressed material can have a higher affinity for activin B ligand than that reported for an ActRIIA-hFc fusion protein expressed in human 293 cells (del Re *et al.*, J Biol Chem. 2004 Dec 17;279(51):53126-35). Additionally, without being bound by theory, the use of the TPA leader sequence provided greater production than other leader sequences and, unlike ActRIIA-Fc expressed with a native leader, may provide a highly pure N-terminal sequence. Use of the native leader sequence may result in two major species of ActRIIA-Fc, each having a different N-terminal sequence.

8.5.2 INHIBITORS OF ACTRIIB SIGNALING

[00218] As used herein, the term "ActRIIB" refers to a family of activin receptor type IIB (ActRIIB) proteins from any species and variants derived from such ActRIIB proteins by mutagenesis or other modification. Reference to ActRIIB herein is understood to be a reference to any one of the currently identified forms of the receptor. Members of the ActRIIB family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

[00219] ActRIIB signaling inhibitors to be used in the compositions and methods described herein include, without limitation, activin-binding soluble ActRIIB polypeptides; antibodies that bind to activin (particularly the activin A or B subunits, also referred to as β_A or β_B) and disrupt ActRIIB binding; antibodies that bind to ActRIIB and disrupt activin binding; non-antibody proteins selected for activin or ActRIIB binding; and randomized peptides selected for activin or ActRIIB binding, which can be conjugated to an Fc domain.

[00220] In certain embodiments, two or more different proteins (or other moieties) with activin or ActRIIB binding activity, especially activin binders that block the type I (*e.g.*, a soluble type I activin receptor) and type II (*e.g.*, a soluble type II activin receptor) binding sites, respectively, may be linked together to create a bifunctional or multifunctional binding molecule that inhibits ActRIIB and thus can be used in the compositions and methods described herein include. In certain embodiments, Activin-ActRIIB signaling axis antagonists that inhibit ActRIIB include nucleic acid aptamers, small molecules and other agents are used in the compositions and methods described herein include.

8.5.2.1 ActRIIB Signaling Inhibitors Comprising ActRIIB Polypeptides

[00221] As used herein, the term "ActRIIB polypeptide" refers to polypeptides comprising any naturally occurring polypeptide of an ActRIIB family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. For example, ActRIIB polypeptides include polypeptides derived from the sequence of any known ActRIIB receptor having a sequence at least about 80% identical to the sequence of an ActRIIB polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. For example, an ActRIIB polypeptide may bind to and inhibit the function of an ActRIIB protein and/or activin. An example of an ActRIIB polypeptide includes the human ActRIIB precursor polypeptide (SEQ ID NO:16 or SEQ ID NO:28). With respect to the ActRIIB precursor polypeptide whose amino acid sequence is depicted as SEQ ID NO:16 or SEQ ID NO:28 (*i.e.,* the human ActRIIB precursor polypeptide), the signal peptide of the ActRIIB precursor polypeptide is located at amino acids 1 to 18; the extracellular domain is located at amino acids 19 to 134 and the potential N-linked glycosylation sites are located at amino acid positions 42 and 65. The nucleic acid sequence encoding the human ActRIIB precursor polypeptide of SEQ ID NO:16 is disclosed as SEQ ID NO:19 (SEQ ID NO:19 provides an alanine at the codon corresponding to amino acid position 64, but could be readily modified by one of skill in the art using methods known in the art to provide an arginine at the

codon corresponding to amino acid position 64 instead). *See* Table 21 for a description of the sequences.

[00222] The numbering of amino acids for all of the ActRIIB-related polypeptides described herein is based on the amino acid numbering for SEQ ID NO:16 and SEQ ID NO:28 (which only differ in the amino acid expressed at position 64), unless specifically designated otherwise. For example, if an ActRIIB polypeptide is described as having a substitution/mutation at amino acid position 79, then it is to be understood that position 79 refers to the 79th amino acid in SEQ ID NO:16 or SEQ ID NO:28, from which the ActRIIB polypeptide is derived. Likewise, if an ActRIIB polypeptide is described as having an alanine or an arginine at amino acid position 64, then it is to be understood that position 64 refers to the 64th amino acid in SEQ ID NO:16 or SEQ ID NO:28, from which the ActRIIB polypeptide is derived.

[00223] In certain embodiments, the inhibitors of ActRIIB signaling used in the compositions and methods described herein comprise polypeptides comprising an activin-binding domain of ActRIIB. In some embodiments, the activin-binding domains of ActRIIB comprise the extracellular domain of ActRIIB, or a portion thereof. In specific embodiments, the extracellular domain or portion thereof of ActRIIB is soluble. Illustrative modified forms of ActRIIB polypeptides are disclosed in U.S. Patent Application Publication Nos. 20090005308 and 20100068215, the disclosures of which are incorporated herein by reference in their entireties.

[00224] In specific embodiments, the ActRIIB polypeptides used in the compositions and methods described herein are soluble ActRIIB polypeptides. The term "soluble ActRIIB polypeptide" generally refers to polypeptides comprising an extracellular domain of an ActRIIB protein, including any naturally occurring extracellular domain of an ActRIIB protein as well as any variants thereof (including mutants, fragments and peptidomimetic forms). Soluble ActRIIB polypeptides can bind to activin; however, the wild type ActRIIB protein does not exhibit significant selectivity in binding to activin versus GDF8/11. In certain embodiments, altered forms of ActRIIB with different binding properties can be used in the methods provided herein. Such altered forms are disclosed, *e.g.*, in international patent application publication Nos. WO 2006/012627 and WO 2010/019261, the disclosures of which are incorporated herein by reference in their entireties. Native or altered ActRIIB proteins may be given added specificity for activin by coupling them with a second, activin-selective binding agent. Exemplary soluble

ActRIIB polypeptides include the extracellular domain of a human ActRIIB polypeptide (*e.g.*, SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43).

[00225] An Fc fusion protein having the ActRIIB extracellular sequence disclosed by Hilden *et al.* (Blood, 1994, 83(8):2163-70), which has an alanine at the position corresponding to amino acid 64 of the ActRIIB precursor amino acid sequence, *i.e.*, SEQ ID NO: 16 (herein referred to as "A64"), has been demonstrated to possess a relatively low affinity for activin and GDF-11. By contrast, an Fc fusion protein with an arginine at position 64 of the ActRIIB precursor amino acid sequence (herein referred to as "R64") has an affinity for activin and GDF-11 in the low nanomolar to high picomolar range (*see, e.g.*, U.S. Patent Application Publication No. 20100068215, the disclosure of which is herein incorporated in its entirety). An ActRIIB precursor amino acid sequence with an arginine at position 64 is presented in SEQ ID NO:28. As such, in certain embodiments, the ActRIIB polypeptides used in accordance with the compositions and methods described herein may comprise either (i) an alanine at the position corresponding to amino acid 64 of the ActRIIB precursor amino acid sequence, *i.e.,* SEQ ID NO: 16; or (ii) an arginine at position 64 of the ActRIIB precursor amino acid sequence, *i.e.,* SEQ ID NO: 28. In other embodiments, the ActRIIB polypeptides used in accordance with the compositions and methods described herein may comprise an amino acid that is not alanine or arginine at the position corresponding to amino acid 64 of the ActRIIB precursor amino acid sequence, *i.e.,* SEQ ID NO: 16 or SEQ ID NO:28.

[00226] It has been shown that a deletion of the proline knot at the C-terminus of the extracellular domain of ActRIIB reduces the affinity of the receptor for activin (*see, e.g.*, Attisano *et al.*, Cell, 1992, 68(1):97-108). An ActRIIB-Fc fusion protein containing amino acids 20-119 of SEQ ID NO: 28 (*i.e.*, SEQ ID NO:32), "ActRIIB(20-119)-Fc" has reduced binding to GDF-11 and activin relative to an ActRIIB-Fc fusion protein containing amino acids 20-134 of SEQ ID NO: 28 (*i.e.*, SEQ ID NO:31), "ActRIIB(20-134)-Fc", which includes the proline knot region and the complete juxtamembrane domain. However, an ActRIIB-Fc fusion protein containing amino acids 20-129 of SEQ ID NO: 28, "ActRIIB(20-129)-Fc" retains similar but somewhat reduced activity relative to the non-truncated extracellular domain of ActRIIB, even though the proline knot region is disrupted. Thus, ActRIIB polypeptides comprising extracellular domains that stop at amino acid 134, 133, 132, 131, 130 and 129 of SEQ ID NO: 28 (or SEQ ID NO:16) are all expected to be active, but constructs stopping at amino acid 134 or 133 may be most active. Similarly, mutations at any of residues 129-134 are not expected to

alter ligand binding affinity by large margins, as indicated by the fact that mutations of P129 and P130 of SEQ ID NO: 28 do not substantially decrease ligand binding. Therefore, the ActRIIB polypeptides used in accordance with the methods and compositions described herein may end as early as amino acid 109 (*i.e.*, the final cysteine) of SEQ ID NO:28 (or SEQ ID NO:16), however, forms ending at or between amino acid positions 109 and 119 of SEQ ID NO:28 (or SEQ ID NO:16) are expected to have reduced ligand binding ability.

[00227] Amino acid 29 of SEQ ID NO:16 and SEQ ID NO:28 represents the initial cysteine in the ActRIIB precursor sequence. It is expected that an ActRIIB polypeptide beginning at amino acid 29 of the N-terminus of SEQ ID NO:16 or SEQ ID NO:28, or before these amino acid positions, will retain ligand binding activity. An alanine to asparagine mutation at position 24 of SEQ ID NO:16 or SEQ ID NO:28 introduces an N-linked glycosylation sequence without substantially affecting ligand binding. This confirms that mutations in the region between the signal cleavage peptide and the cysteine cross-linked region, corresponding to amino acids 20-29 of SEQ ID NO:16 or SEQ ID NO:28, are well tolerated. In particular, ActRIIB polypeptides beginning at amino acid position 20, 21, 22, 23 and 24 of SEQ ID NO:16 or SEQ ID NO:28 will retain activity, and ActRIIB polypeptides beginning at amino acid positions 25, 26, 27, 28 and 29 of SEQ ID NO:16 or SEQ ID NO:28 are also expected to retain activity. An ActRIIB polypeptide beginning at amino acid position 22, 23, 24 or 25 of SEQ ID NO:16 or SEQ ID NO:28 will have the most activity.

[00228] Taken together, the active portions (*i.e.*, ActRIIB polypeptides) of the ActRIIB precursor protein (*i.e.*, SEQ ID NO:16 or SEQ ID NO:28) to be used in accordance with the methods and compositions described herein will generally comprise amino acids 29-109 of SEQ ID NO:16 or SEQ ID NO:28, and such ActRIIB polypeptides may, for example, begin at a residue corresponding to any one of amino acids 19-29 of SEQ ID NO:16 or SEQ ID NO:28 and end at a position corresponding to any one of amino acids 109-134 of SEQ ID NO:16 or SEQ ID NO:28. Specific examples of ActRIIB polypeptides encompassed herein include those that begin at an amino acid position from 19-29, 20-29 or 21-29 of SEQ ID NO:16 or SEQ ID NO:28 and end at an amino acid position from 119-134, 119-133 or 129-134, 129-133 of SEQ ID NO:16 or SEQ ID NO:28. Other specific examples of ActRIIB polypeptides encompassed herein include those that begin at an amino acid position from 20-24 (or 21-24, or 22-25) of SEQ ID NO:16 or SEQ ID NO:28 and end at an amino acid position from 109-134 (or 109-133), 119-134 (or 119-133) or 129-134 (or 129-133) of SEQ ID NO:16 or SEQ ID NO:28. Variant

ActRIIB polypeptides falling within these ranges are also contemplated, particularly those having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or sequence homology to the corresponding portion of SEQ ID NO:16 or SEQ ID NO:28.

[00229] In certain embodiments, the inhibitors of ActRIIB signaling used in the compositions and methods described herein comprise a truncated form of an extracellular domain of ActRIIB. The truncation can be at the carboxy terminus and/or the amino terminus of the ActRIIB polypeptide. In certain embodiments, the truncation can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids long relative to the mature ActRIIB polypeptide extracellular domain. In certain embodiments, the truncation can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 N-terminal amino acids of the mature ActRIIB polypeptide extracellular domain. In certain embodiments, the truncation can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 C-terminal amino acids of the mature ActRIIB polypeptide extracellular domain. For example, truncated forms of ActRIIB include polypeptides with amino acids 20-119; 20-128; 20-129; 20-130; 20-131; 20-132; 20-133; 20-134; 20-131; 21-131; 22-131; 23-131; 24-131; and 25-131, wherein the amino acid positions refer to the amino acid positions in SEQ ID NO:16 or SEQ ID NO:28.

[00230] Additional exemplary truncated forms of ActRIIB include (i) polypeptides beginning at amino acids at any of amino acids 21-29 of SEQ ID NO:16 or SEQ ID NO:28 (optionally beginning at 22-25 of SEQ ID NO:16 or SEQ ID NO:28) and ending at any of amino acids 109-134 of SEQ ID NO:16 or SEQ ID NO:28; (ii) polypeptides beginning at any of amino acids 20-29 of SEQ ID NO:16 or SEQ ID NO:28 (optionally beginning at 22-25 of SEQ ID NO:16 or SEQ ID NO:28) and ending at any of amino acids 109-133 of SEQ ID NO:16 or SEQ ID NO:28; (iii) polypeptides beginning at any of amino acids 20-24 of SEQ ID NO:16 or SEQ ID NO:28 (optionally beginning at 22-25 of SEQ ID NO:16 or SEQ ID NO:28) and ending at any of amino acids 109-133 of SEQ ID NO:16 or SEQ ID NO:28; (iv) polypeptides beginning at any of amino acids 21-24 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 109-134 of SEQ ID NO:16 or SEQ ID NO:28; (v) polypeptides beginning at any of amino acids 20-24 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 118-133 of SEQ ID NO:16 or SEQ ID NO:28; (vi) polypeptides beginning at any of amino acids 21-24 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 118-134 of SEQ ID NO:16 or SEQ

ID NO:28; (vii) polypeptides beginning at any of amino acids 20-24 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 128-133 of SEQ ID NO:16 or SEQ ID NO:28; (viii) polypeptides beginning at any of amino acids 20-24 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 128-133 of SEQ ID NO:16 or SEQ ID NO:28; (ix) polypeptides beginning at any of amino acids 21-29 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 118-134 of SEQ ID NO:16 or SEQ ID NO:28; (x) polypeptides beginning at any of amino acids 20-29 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 118-133 of SEQ ID NO:16 or SEQ ID NO:28; (xi) polypeptides beginning at any of amino acids 21-29 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 128-134 of SEQ ID NO:16 or SEQ ID NO:28; and (xii) polypeptides beginning at any of amino acids 20-29 of SEQ ID NO:16 or SEQ ID NO:28 and ending at any of amino acids 128-133 of SEQ ID NO:16 or SEQ ID NO:28. In a specific embodiment, an ActRIIB polypeptides comprises, consists essentially of, or consists of, an amino acid sequence beginning at amino acid position 25 of SEQ ID NO:16 or SEQ ID NO:28 and ending at amino acid position 131 of SEQ ID NO:16 or SEQ ID NO:28. In another specific embodiment, an ActRIIB polypeptide consists of, or consists essentially of, the amino acid sequence of SEQ ID NO:17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, or 43.

[00231] Any of the ActRIIB polypeptides used in the compositions and methods described herein may be produced as a homodimer. Any of the ActRIIB polypeptides used in the compositions and methods described herein may be formulated as a fusion protein having a heterologous portion that comprises a constant region from an IgG heavy chain, such as an Fc domain. Any of the ActRIIB polypeptides used in the compositions and methods described herein may comprise an acidic amino acid at the position corresponding to position 79 of SEQ ID NO:16 or SEQ ID NO:28, optionally in combination with one or more additional amino acid substitutions, deletions or insertions relative to SEQ ID NO:16 or SEQ ID NO:28.

[00232] In specific embodiments, the inhibitors of ActRIIB signaling used in the compositions and methods described herein comprise an extracellular domain of ActRIIB with one or more amino acid substitutions/mutations. Such an amino acid substitution/mutation can be, for example, an exchange from the leucine at amino acid position 79 of SEQ ID NO:16 or SEQ ID NO:28 to an acidic amino acid, such as aspartic acid or glutamic acid. For example, position L79 of SEQ ID NO:16 or SEQ ID NO:28 may be altered in ActRIIB extracellular domain polypeptides to confer altered activin-myostatin (GDF-11) binding properties. L79A and

L79P mutations reduce GDF-11 binding to a greater extent than activin binding. L79E and L79D mutations retain GDF-11 binding, while demonstrating greatly reduced activin binding.

[00233] In certain embodiments, the inhibitors of ActRIIB signaling used in the compositions and methods described herein comprise a truncated form of an ActRIIB extracellular domain that also carries an amino acid substitution, *e.g.*, an exchange from the leucine at amino acid position 79 of SEQ ID NO:16 or SEQ ID NO:28 to an acidic amino acid, such as aspartic acid or glutamic acid. In a specific embodiment, the truncated form of an extracellular domain of ActRIIB polypeptide that also carries an amino acid substitution used in the compositions and methods described herein is SEQ ID NO:23. Forms of ActRIIB that are truncated and/or carry one or more amino acid substitutions can be linked to an Fc domain of an antibody as discussed above.

[00234] Functionally active fragments of ActRIIB polypeptides can be obtained, for example, by screening polypeptides recombinantly produced from the corresponding fragment of the nucleic acid encoding an ActRIIB polypeptide. In addition, fragments can be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. As will be recognized by one of skill in the art, other techniques, including for example various resins and solution phase systems for peptide synthesis can be used to synthesize peptidyl fragments described herein. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those peptidyl fragments that can function as antagonists (inhibitors) of ActRIIB protein or signaling mediated by activin.

[00235] In addition, functionally active variants of ActRIIB polypeptides can be obtained, for example, by screening libraries of modified polypeptides recombinantly produced from the corresponding mutagenized nucleic acids encoding an ActRIIB polypeptide. The variants can be produced and tested to identify those that can function as antagonists (inhibitors) of ActRIIB protein or signaling mediated by activin. In certain embodiments, a functional variant of the ActRIIB polypeptides comprises an amino acid sequence that is at least 75% identical to an amino acid sequence selected from SEQ ID NO:17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43. In certain embodiments, the functional variant has an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NO:17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43.

[00236] Functional variants may be generated, for example, by modifying the structure of an ActRIIB polypeptide for such purposes as enhancing therapeutic efficacy, or stability (*e.g.*, ex

vivo shelf life and resistance to proteolytic degradation in vivo). Such modified ActRIIB polypeptides when selected to retain activin binding, are considered functional equivalents of the naturally-occurring ActRIIB polypeptides. Modified ActRIIB polypeptides can also be produced, for instance, by amino acid substitution, deletion, or addition. For instance, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*e.g.*, conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of an ActRIIB polypeptide results in a functional homolog can be readily determined by assessing the ability of the variant ActRIIB polypeptide to produce a response in cells in a fashion similar to the wild-type ActRIIB polypeptide.

[00237] ActRIIB polypeptide mutants, particularly sets of combinatorial mutants of an ActRIIB polypeptide, as well as truncation mutants; pools of combinatorial mutants are especially useful for identifying functional variant sequences can be used in the methods and compositions described herein. The purpose of screening such combinatorial libraries may be to generate, for example, ActRIIB polypeptide variants which can act as either agonists or antagonist, or alternatively, which possess novel activities all together.

[00238] It has been demonstrated that the ligand binding pocket of ActRIIB is defined by residues Y31, N33, N35, L38 through T41, E47, E50, Q53 through K55, L57, H58, Y60, S62, K74, W78 through N83, Y85, R87, A92, and E94 through F101 of SEQ ID NO:16 or SEQ ID NO:28. At these positions, it is expected that conservative mutations will be tolerated, although a K74A mutation is well-tolerated, as are R40A, K55A, F82A and mutations at position L79. R40 is a K in Xenopus, indicating that basic amino acids at this position will be tolerated. Q53 is R in bovine ActRIIB and K in Xenopus ActRIIB, and therefore amino acids including R, K, Q, N and H will be tolerated at this position. Thus, a general formula for an ActRIIB polypeptide for use in the methods and compositions described herein is one that comprises amino acids 29-109 of SEQ ID NO:16 or SEQ ID NO:28, but optionally beginning at an amino acid position ranging from 20-24 or 22-25 of SEQ ID NO:16 or SEQ ID NO:28 and ending at an amino acid position ranging from 129-134 of SEQ ID NO:16 or SEQ ID NO:28, and comprising no more than 1, 2, 5, or 15 conservative amino acid changes in the ligand binding pocket, and zero, one or more non-conservative alterations at amino acid positions 40, 53, 55, 74, 79 and/or 82 of SEQ ID

NO:16 or SEQ ID NO:28 in the ligand binding pocket. Such an ActRIIB polypeptide may retain greater than 80%, 90%, 95% or 99% sequence identity or sequence homology to the sequence of amino acids 29-109 of SEQ ID NO:16 or SEQ ID NO:28. Sites outside the binding pocket, at which variability may be particularly well tolerated, include the amino and carboxy termini of the extracellular domain of ActRIIB, and positions 42-46 and 65-73. An asparagine to alanine alteration at position 65 of SEQ ID NO:16 or SEQ ID NO:28 (N65A) actually improves ligand binding in the A64 background, and is thus expected to have no detrimental effect on ligand binding in the R64 background. This change probably eliminates glycosylation at N65 in the A64 background, thus demonstrating that a significant change in this region is likely to be tolerated. While an R64A change is poorly tolerated, R64K is well-tolerated, and thus another basic residue, such as H may be tolerated at position 64.

[00239] As a specific example of an ActRIIB polypeptide with a mutation in the ligand binding domain, the positively-charged amino acid residue Asp (D80) of the ligand-binding domain of ActRIIB can be mutated to a different amino acid residue such that the variant ActRIIB polypeptide preferentially binds to GDF8, but not activin. In a specific embodiment, the D80 residue is changed to an amino acid residue selected from the group consisting of: an uncharged amino acid residue, a negative amino acid residue, and a hydrophobic amino acid residue. As a further specific example, the hydrophobic residue L79 can be altered to the acidic amino acids aspartic acid or glutamic acid to greatly reduce activin binding while retaining GDF11 binding. As will be recognized by one of skill in the art, most of the described mutations, variants or modifications may be made at the nucleic acid level or, in some cases, by post translational modification or chemical synthesis. Such techniques are well known in the art.

[00240] In specific embodiments, the inhibitors of ActRIIB signaling used in the compositions and methods described herein comprise a conjugate/fusion protein comprising an extracellular domain (*e.g.*, an activin-binding domain) of an ActRIIB receptor linked to an Fc portion of an antibody. Such conjugate/fusion proteins may comprise any of the ActRIIB polypeptides disclosed herein (*e.g.*, any of SEQ ID NOS:17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, or 43), any ActRIIB polypeptides known in the art, or any ActRIIB polypeptides generated using methods known in the art and/or provided herein.

[00241] In certain embodiments, the extracellular domain is linked to an Fc portion of an antibody via a linker, *e.g.*, a peptide linker. Exemplary linkers include short polypeptide sequences such as 2-10, 2-5, 2-4, 2-3 amino acid residues (*e.g.*, glycine residues), such as, for

example, a Gly-Gly-Gly linker. In a specific embodiment, the linker comprises the amino acid sequence Gly-Gly-Gly (GGG). In another specific embodiment, the linker comprises the amino acid sequence Thr-Gly-Gly-Gly (TGGG). Optionally, the Fc domain has one or more mutations at residues such as Asp-265, lysine 322, and Asn-434. In certain cases, the mutant Fc domain having one or more of these mutations (*e.g.*, an Asp-265 mutation) has a reduced ability to bind to the Fcγ receptor relative to a wild-type Fc domain. In other cases, the mutant Fc domain having one or more of these mutations (*e.g.*, an Asn-434 mutation) has an increased ability to bind to the MHC class I- related Fc-receptor (FcRN) relative to a wild-type Fc domain. Exemplary fusion proteins comprising a soluble extracellular domain of ActRIIB fused to an Fc domain are set forth in SEQ ID NOs:20, 21, 24, 25, 34, 35, 38, 39, 40, 41, 44, 46, and 47.

[00242] In a specific embodiment, the ActRIIB signaling inhibitors used in the compositions and methods described herein comprise the extracellular domain of ActRIIB, or a portion thereof, linked to an Fc portion of an antibody, wherein said ActRIIB signaling inhibitor comprises an amino acid sequence that is at least 75% identical to an amino acid sequence selected from SEQ ID NOs:20, 21, 24, 25, 34, 35, 38, 39, 40, 41, 44, 46, and 47. In another specific embodiment, the ActRIIB signaling inhibitors used in the compositions and methods described herein comprise the extracellular domain of ActRIIB, or a portion thereof, linked to an Fc portion of an antibody, wherein said ActRIIB signaling inhibitor comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOS:20, 21, 24, 25, 34, 35, 38, 39, 40, 41, 44, 46, and 47.

[00243] In a specific embodiment, the ActRIIB signaling inhibitor to be used in the compositions and methods described herein is a fusion protein between the extracellular domain of the human ActRIIB receptor and the Fc portion of IgG1. In another specific embodiment, the ActRIIB signaling inhibitor to be used in the compositions and methods described herein is a fusion protein between a truncated extracellular domain of the human ActRIIB receptor and the Fc portion of IgG1. In another specific embodiment, the ActRIIB signaling inhibitor to be used in the compositions and methods described herein is a fusion protein between a truncated extracellular domain of the human ActRIIB receptor and the Fc portion of IgG1, wherein the truncated extracellular domain of the human ActRIIB receptor possesses an amino acid substitution at the amino acid position corresponding to amino acid 79 of SEQ ID NO:16 or SEQ ID NO:28. In one embodiment, the amino acid substitution at the amino acid position

corresponding to amino acid 79 of SEQ ID NO:16 or SEQ ID NO:28 is substitution of Leucine for Aspartic Acid (*i.e.*, an L79D mutation).

[00244] In a specific embodiment, the ActRIIB signaling inhibitor to be used in the compositions and methods described herein is SEQ ID NO:24 or 25, which represents a fusion protein between the extracellular domain of the human ActRIIB receptor and the Fc portion of IgG1, wherein said ActRIIB extracellular domain comprises amino acids 25-131 of SEQ ID NO:28 with an L79D mutation. The nucleic acid sequence encoding the ActRIIB-Fc fusion protein of SEQ ID NO:24 is presented in SEQ ID NO:45.

[00245] In another specific embodiment, the ActRIIB signaling inhibitor to be used in the compositions and methods described herein is SEQ ID NO:34 or 35, which represents a fusion protein between the extracellular domain of the human ActRIIB receptor and the Fc portion of IgG1, wherein said ActRIIB extracellular domain comprises amino acids 25-131 of SEQ ID NO:16 with an L79D mutation.

[00246] Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine (or asparagine-X-serine) (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the wild-type ActRIIB polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on an ActRIIB polypeptide is by chemical or enzymatic coupling of glycosides to the ActRIIB polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. These methods are described in International Patent Application No. WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston (1981) CRC Crit. Rev. Biochem., pp. 259-306, incorporated by reference herein. Removal of one or more carbohydrate moieties present on an ActRIIB polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of the ActRIIB polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent

compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Chemical deglycosylation is further described by Hakimuddin *et al.* (1987) Arch. Biochem. Biophys. 259:52 and by Edge *et al.* (1981) Anal. Biochem. 118:131. Enzymatic cleavage of carbohydrate moieties on ActRIIB polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* (1987) Meth. Enzymol. 138:350. The sequence of an ActRIIB polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, ActRIIB proteins for use in humans will be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other expression systems, such as other mammalian expression cell lines, yeast cell lines with engineered glycosylation enzymes and insect cells, are expected to be useful as well.

[00247] In specific embodiments, mutated ActRIIB polypeptides comprising the addition of a further N-linked glycosylation site (N-X-S/T) that increases the serum half-life of an ActRIIB-Fc fusion protein, relative to the ActRIIB(R64)-Fc form can be used in the methods and compositions described herein. In a specific embodiment, introduction of an asparagine at position 24 of SEQ ID NO:16 or SEQ ID NO:28 (A24N) results in the creation of an NXT sequence that confers a longer half-life. Other NX(T/S) sequences can be found at 42-44 (NQS) and 65-67 (NSS), although the latter may not be efficiently glycosylated with the R at position 64 (*i.e.*, in R64 polypeptides). N-X-S/T sequences may be generally introduced at positions outside the ligand binding pocket of ActRIIB, which is detailed above. Particularly suitable sites for the introduction of non-endogenous N-X-S/T sequences include amino acids 20-29, 20-24, 22-25, 109-134, 120-134 or 129-134 of SEQ ID NO:16 or SEQ ID NO:28. N-X-S/T sequences may also be introduced into the linker between the ActRIIB sequence and the Fc or other fusion component. Such a site may be introduced with minimal effort by introducing an N in the correct position with respect to a pre-existing S or T, or by introducing an S or T at a position corresponding to a pre-existing N. Thus, desirable alterations that would create an N-linked glycosylation site are: A24N, R64N, S67N (possibly combined with an N65A alteration), E106N, R112N, G120N, E123N, P129N, A132N, R112S and R112T (with all amino acid positions corresponding to the positions they can be found in SEQ ID NO:16 or SEQ ID NO:28). Any S that is predicted to be glycosylated may be altered to a T without creating an

immunogenic site, because of the protection afforded by the glycosylation. Likewise, any T that is predicted to be glycosylated may be altered to an S. Thus the alterations S67T and S44T are encompassed herein. Likewise, in an A24N variant, an S26T alteration may be used. Accordingly, an ActRIIB polypeptide may include one or more additional, non-endogenous N-linked glycosylation consensus sequences.

[00248] A variety of screening assays may be used to evaluate ActRIIB polypeptide variants. For example, an ActRIIB polypeptide variant may be screened for ability to bind to an ActRIIB ligand, to prevent binding of an ActRIIB ligand to an ActRIIB polypeptide or to interfere with signaling caused by an ActRIIB ligand. The activity of an ActRIIB polypeptide or its variants may also be tested in a cell-based or in vivo assay.

[00249] Combinatorially-derived variants can be generated which have a selective or generally increased potency relative to a naturally occurring ActRIIB polypeptide. Likewise, mutagenesis can give rise to variants which have intracellular half-lives dramatically different than the corresponding wild-type ActRIIB polypeptide. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other cellular processes which result in destruction of, or otherwise inactivation of a native ActRIIB polypeptide. Such variants, and the genes which encode them, can be utilized to alter ActRIIB polypeptide levels by modulating the half-life of the ActRIIB polypeptides. For instance, a short half-life can give rise to more transient biological effects and can allow tighter control of recombinant ActRIIB polypeptide levels within the subject. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter the half-life of the protein.

[00250] A combinatorial library may be produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential ActRIIB polypeptide sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential ActRIIB polypeptide nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display).

[00251] There are many ways by which the library of potential homologs can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art (*see* for example, Narang, S A (1983) Tetrahedron 39:3; Itakura *et al.*, (1981)

Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp 273-289; Itakura *et al.*, (1984) Annu. Rev. Biochem. 53:323; Itakura *et al.*, (1984) Science 198:1056; Ike *et al.*, (1983) Nucleic Acid Res. 11:477). Such techniques have been employed in the directed evolution of other proteins (*see*, for example, Scott *et al.*, (1990) Science 249:386-390; Roberts *et al.*, (1992) PNAS USA 89:2429-2433; Devlin *et al.*, (1990) Science 249: 404-406; Cwirla *et al.*, (1990) PNAS USA 87: 6378-6382; as well as U.S. Pat. Nos. 5,223,409, 5,198,346, and 5,096,815).

[00252] Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, ActRIIB polypeptide variants can be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis and the like (Ruf *et al.*, (1994) Biochemistry 33:1565-1572; Wang *et al.*, (1994) J. Biol. Chem. 269:3095-3099; Balint *et al.*, (1993) Gene 137:109-118; Grodberg *et al.*, (1993) Eur. J. Biochem. 218:597-601; Nagashima *et al.*, (1993) J. Biol. Chem. 268:2888-2892; Lowman *et al.*, (1991) Biochemistry 30:10832-10838; and Cunningham *et al.*, (1989) Science 244:1081-1085), by linker scanning mutagenesis (Gustin *et al.*, (1993) Virology 193:653-660; Brown *et al.*, (1992) Mol. Cell Biol. 12:2644-2652; McKnight *et al.*, (1982) Science 232:316); by saturation mutagenesis (Meyers *et al.*, (1986) Science 232:613); by PCR mutagenesis (Leung *et al.*, (1989) Method Cell Mol Biol 1:11-19); or by random mutagenesis, including chemical mutagenesis, etc. (Miller *et al.*, (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, N.Y.; and Greener *et al.*, (1994) Strategies in Mol Biol 7:32-34). Linker scanning mutagenesis, particularly in a combinatorial setting, is an attractive method for identifying truncated (bioactive) forms of ActRIIB polypeptides.

[00253] A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and, for that matter, for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of ActRIIB polypeptides. The most widely used techniques for screening large gene libraries typically comprises cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include activin binding assays and activin-mediated cell signaling assays.

[00254] In certain embodiments, ActRIIB polypeptides used in the methods and compositions described herein may further comprise post-translational modifications in addition to any that are naturally present in the ActRIIB polypeptides. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, the modified ActRIIB polypeptides may contain non-amino acid elements, such as polyethylene glycols, lipids, poly- or mono-saccharide, and phosphates. Effects of such non-amino acid elements on the functionality of an ActRIIB polypeptide may be tested by any method known to the skilled artisan. When an ActRIIB polypeptide is produced in cells by cleaving a nascent form of the ActRIIB polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (such as CHO, HeLa, MDCK, 293, W138, NIH-3T3 or HEK293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the ActRIIB polypeptides.

[00255] In certain aspects, functional variants or modified forms of the ActRIIB polypeptides include fusion proteins having at least a portion of the ActRIIB polypeptides and one or more fusion domains. Well known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt-conjugated resins are used. Many of such matrices are available in "kit" form, such as the Pharmacia GST purification system and the QIAexpressTM system (Qiagen) useful with (HIS6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ActRIIB polypeptides. Examples of such detection domains include the various fluorescent proteins (*e.g.*, GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus hemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for Factor Xa or Thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion

domain by subsequent chromatographic separation. In certain preferred embodiments, an ActRIIB polypeptide is fused with a domain that stabilizes the ActRIIB polypeptide in vivo (a "stabilizer" domain). By "stabilizing" is meant anything that increases serum half life, regardless of whether this is because of decreased destruction, decreased clearance by the kidney, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin are known to confer desirable pharmacokinetic properties on a wide range of proteins. Likewise, fusions to human serum albumin can confer desirable properties. Other types of fusion domains that may be selected include multimerizing (*e.g.*, dimerizing, tetramerizing) domains and functional domains (that confer an additional biological function, such as further stimulation of bone growth or muscle growth, as desired).

[00256] It is understood that different elements of the fusion proteins may be arranged in any manner that is consistent with the desired functionality. For example, an ActRIIB polypeptide may be placed C-terminal to a heterologous domain, or, alternatively, a heterologous domain may be placed C-terminal to an ActRIIB polypeptide. The ActRIIB polypeptide domain and the heterologous domain need not be adjacent in a fusion protein, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains.

[00257] In certain embodiments, the ActRIIB polypeptides used in the methods and compositions described herein contain one or more modifications that are capable of stabilizing the ActRIIB polypeptides. For example, such modifications enhance the in vitro half life of the ActRIIB polypeptides, enhance circulatory half life of the ActRIIB polypeptides or reduce proteolytic degradation of the ActRIIB polypeptides. Such stabilizing modifications include, but are not limited to, fusion proteins (including, for example, fusion proteins comprising an ActRIIB polypeptide and a stabilizer domain), modifications of a glycosylation site (including, for example, addition of a glycosylation site to an ActRIIB polypeptide), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from an ActRIIB polypeptide). In the case of fusion proteins, an ActRIIB polypeptide is fused to a stabilizer domain such as an IgG molecule (*e.g.*, an Fc domain). As used herein, the term "stabilizer domain" not only refers to a fusion domain (*e.g.*, Fc) as in the case of fusion proteins, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous polymer, such as polyethylene glycol.

[00258] In certain embodiments, the methods and compositions described herein use isolated or purified ActRIIB polypeptides, *i.e.*, ActRIIB polypeptides which are isolated from, or otherwise substantially free of, other proteins can be used with the methods and compositions described herein. ActRIIB polypeptides will generally be produced by expression from recombinant nucleic acids.

[00259] In certain aspects, the ActRIIB polypeptides used in the methods and compositions described herein are encoded by isolated and/or recombinant nucleic acids, including fragments, functional variants and fusion proteins disclosed herein. For example, SEQ ID NO:19 encodes the naturally occurring human ActRIIB precursor polypeptide. The subject nucleic acids may be single-stranded or double stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making ActRIIB polypeptides or as direct therapeutic agents (*e.g.*, in a gene therapy approach).

[00260] In certain aspects, the nucleic acids that can be used to produce ActRIIB polypeptides suitable for use in the methods and compositions described herein are further understood to include nucleic acids that are variants of SEQ ID NO: 19 as well as variants of those nucleic acid sequences that encode soluble ActRIIB polypeptides (*e.g.*, nucleic acids that encode SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43). Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants.

[00261] In certain embodiments, the isolated or recombinant nucleic acid sequences that can be used to produce ActRIIB polypeptides suitable for use in the methods and compositions described herein are at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO:19 or those nucleic acid sequences that encode soluble ActRIIB polypeptides (*e.g.*, nucleic acids that encode SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43). One of ordinary skill in the art will appreciate that nucleic acid sequences complementary to SEQ ID NO:19 or those nucleic acid sequences that encode soluble ActRIIB polypeptides (*e.g.*, nucleic acids that encode SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43), and variants of SEQ ID NO:19 or those nucleic acid sequences that encode soluble ActRIIB polypeptides (*e.g.*, nucleic acids that encode SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43) can be used with the methods and compositions described herein. In further embodiments, the nucleic acid sequences can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

[00262] In other embodiments, nucleic acids that can be used to produce ActRIIB polypeptides suitable for use in the methods and compositions described herein include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequence designated in SEQ ID NO:19 or those nucleic acid sequences that encode soluble ActRIIB polypeptides (*e.g.*, nucleic acids that encode SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43), complement sequence of SEQ ID NO:19 or those nucleic acid sequences that encode soluble ActRIIB polypeptides (*e.g.*, nucleic acids that encode SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43), or fragments thereof. One of ordinary skill in the art will understand that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one can perform the hybridization at 6.0 times sodium chloride/sodium citrate (SSC) at about 45 degree Celsius, followed by a wash of 2.0 times SSC at 50 degree Celsius. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 times SSC at 50 degree Celsius to a high stringency of about 0.2 times SSC at 50 degree Celsius. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22 degree Celsius, to high stringency conditions at about 65 degree Celsius. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In one embodiment, nucleic acids which hybridize under low stringency conditions of 6 times SSC at room temperature followed by a wash at 2 times SSC at room temperature can be used with the methods and compositions described herein.

[00263] Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NO:19 or those nucleic acid sequences that encode soluble ActRIIB polypeptides (*e.g.*, nucleic acids that encode SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43) due to degeneracy in the genetic code can also be used to produce ActRIIB polypeptides suitable for use in the methods and compositions described herein. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of a given species due to natural

allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms can be used with the methods and compositions described herein.

[00264] In certain embodiments, the recombinant nucleic acids that can be used to produce ActRIIB polypeptides suitable for use in the methods and compositions described herein may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art can be used with the methods and compositions described herein. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome. In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

[00265] In certain aspects, the nucleic acids that can be used to produce ActRIIB polypeptides suitable for use in the methods and compositions described herein are provided in an expression vector comprising a nucleotide sequence encoding an ActRIIB polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the ActRIIB polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; Gene Expression Technology: Methods in Enzymology, Academic Press, San Diego, Calif. (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding an ActRIIB polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage

lambda, the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, *e.g.*, Pho5, the promoters of the yeast .alpha.-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

[00266] A recombinant nucleic acid can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression vehicles for production of a recombinant ActRIIB polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as E. coli.

[00267] Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, *see* Molecular Cloning A Laboratory Manual, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a

baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the .beta.-gal containing pBlueBac III).

[00268] In one embodiment, a vector can be designed for production of the ActRIIB polypeptides used in the methods and compositions described herein in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wis.). As will be apparent, the subject gene constructs can be used to cause expression of the subject ActRIIB polypeptides in cells propagated in culture, *e.g.*, to produce proteins, including fusion proteins or variant proteins, for purification.

[00269] Host cells transfected with a recombinant gene including a coding sequence (*e.g.*, SEQ ID NO:19 or those nucleic acid sequences that encode soluble ActRIIB polypeptides (*e.g.*, nucleic acids that encode SEQ ID NOs: 17, 18, 23, 26, 27, 29, 30, 31, 32, 33, 36, 37, 42, and 43)) for one or more of the subject ActRIIB polypeptides can be used to produce ActRIIB polypeptides suitable for use in the methods and compositions described herein. The host cell may be any prokaryotic or eukaryotic cell. For example, an ActRIIB polypeptide may be expressed in bacterial cells such as E. coli, insect cells (*e.g.*, using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art.

[00270] Accordingly, provided herein are methods of producing the ActRIIB polypeptides used in the methods and compositions described herein. For example, a host cell transfected with an expression vector encoding an ActRIIB polypeptide can be cultured under appropriate conditions to allow expression of the ActRIIB polypeptide to occur. The ActRIIB polypeptide may be secreted and isolated from a mixture of cells and medium containing the ActRIIB polypeptide. Alternatively, the ActRIIB polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art. The subject ActRIIB polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the ActRIIB polypeptides and affinity purification with an agent that binds to a domain fused to the ActRIIB polypeptide (*e.g.*, a protein A column may be used to purify an ActRIIB-Fc fusion). In a preferred embodiment,

the ActRIIB polypeptide is a fusion protein containing a domain which facilitates its purification. In a preferred embodiment, purification is achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. As demonstrated herein, ActRIIB -hFc protein was purified to a purity of >98% as determined by size exclusion chromatography and >95% as determined by SDS PAGE. This level of purity was sufficient to achieve desirable effects on bone in mice and an acceptable safety profile in mice, rats and non-human primates.

[00271] In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant ActRIIB polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni2+ metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified ActRIIB polypeptide (*e.g.*, *see* Hochuli *et al.*, (1987) J. Chromatography 411:177; and Janknecht *et al.*, PNAS USA 88:8972).

[00272] Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence (*see*, for example, Current Protocols in Molecular Biology, eds. Ausubel *et al.*, John Wiley & Sons: 1992).

[00273] ActRIIB -Fc fusion protein can be expressed in stably transfected CHO-DUKX Bl 1 cells from a pAID4 vector (SV40 ori/enhancer, CMV promoter), using a tissue plasminogen leader sequence of SEQ ID NO:8. The Fc portion can comprise a human IgGl Fc sequence, as shown in SEQ ID NO:7. In certain embodiments, upon expression, the protein contained has, on

average, between about 1.5 and 2.5 moles of sialic acid per molecule of ActRIIB-Fc fusion protein.

[00274] In certain embodiments, the long serum half-life of an ActRIIB-Fc fusion can be 25-32 days in human subjects. Additionally, the CHO cell expressed material can have a higher affinity for activin B ligand than that reported for an ActRIIB-hFc fusion protein expressed in human 293 cells (del Re *et al.*, J Biol Chem. 2004 Dec 17;279(51):53126-35). Additionally, without being bound by theory, the use of the TPA leader sequence provided greater production than other leader sequences and, unlike ActRIIB-Fc expressed with a native leader, may provide a highly pure N-terminal sequence. Use of the native leader sequence may result in two major species of ActRIIB-Fc, each having a different N-terminal sequence.

8.5.3 OTHER ACTRII RECEPTOR SIGNALING INHIBITORS

[00275] In certain embodiments, the inhibitors of ActRII signaling used in the compositions and methods described herein are nucleic acid compounds.

[00276] Examples of categories of nucleic acid compounds that inhibit ActRII receptors include antisense nucleic acids, siRNA or RNAi constructs and catalytic nucleic acid constructs. A nucleic acid compound may be single- or double-stranded. A double-stranded compound may also include regions of overhang or non-complementarity, where one or the other of the strands is single-stranded. A single-stranded compound may include regions of self-complementarity, meaning that the compound may form a so-called "hairpin" or "stem-loop" structure, with a region of double helical structure.

[00277] In certain embodiments, the nucleic acid compounds that inhibit ActRII receptors may comprise a nucleotide sequence that is complementary to a region consisting of no more than 1000, no more than 500, no more than 250, no more than 100 or no more than 50, 35, 30, 25, 22, 20 or 18 nucleotides of the full-length ActRII receptor nucleic acid sequence or activin nucleic acid sequence (*e.g.*, the nucleic acid sequence of an activin A or activin B subunit, also referred to as β_A or β_B). In specific embodiments, the region of complementarity will be at least 8 nucleotides, and optionally at least 10 or at least 15 nucleotides, and optionally between 15 and 25 nucleotides. A region of complementarity may fall within an intron, a coding sequence or a noncoding sequence of the target transcript, such as the coding sequence portion. Generally, a nucleic acid compound that inhibits an ActRII receptor will have a length of about 8 to about 500 nucleotides or base pairs in length, and optionally the length will be about 14 to about 50 nucleotides. A nucleic acid compound that inhibits an ActRII receptor may be a DNA

(particularly for use as an antisense), an RNA, or an RNA:DNA hybrid. Any one strand may include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA. Likewise, a double stranded nucleic acid compound may be DNA:DNA, DNA:RNA, or RNA:RNA, and any one strand may also include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA.

[00278] The nucleic acid compounds that inhibit an ActRII receptor may include any of a variety of modifications, including one or modifications to the backbone (the sugar-phosphate portion in a natural nucleic acid, including internucleotide linkages) or the base portion (the purine or pyrimidine portion of a natural nucleic acid). In certain embodiments, an antisense nucleic acid compound will have a length of about 15 to about 30 nucleotides and will often contain one or more modifications to improve certain characteristics, such as stability in the serum, stability in a cell, or stability in a place where the compound is likely to be delivered, such as, *e.g.*, the stomach in the case of orally delivered compounds and the lung for inhaled compounds. In the case of an RNAi construct, the strand complementary to the target transcript will generally be RNA or modifications thereof. The other strand may be RNA, DNA, or any other variation. The duplex portion of double stranded or single stranded "hairpin" RNAi construct may, in certain embodiments, have a length of 18 to 40 nucleotides in length and optionally about 21 to 23 nucleotides in length, so long as it serves as a Dicer substrate. Catalytic or enzymatic nucleic acids may be ribozymes or DNA enzymes and may also contain modified forms. In certain embodiments, nucleic acid compounds that inhibit ActRII receptors may inhibit expression of their target by about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more under physiological conditions and at a concentration where a nonsense or sense control has little or no effect. Concentrations for testing the effect of nucleic acid compounds include 1, 5, 10 micromolar, or more.

[00279] In other embodiments, the inhibitors of ActRII signaling used in the compositions and methods described herein are antibodies. Such antibodies include antibodies that bind to activin (particularly the activin A or B subunits, also referred to as βA or βB) and disrupt ActRII receptor binding; and antibodies that bind to ActRII receptor polypeptides (*e.g.*, a soluble ActRIIA or soluble ActRIIB polypeptide) and disrupt activin binding.

[00280] By using immunogens derived from an ActRII receptor polypeptide or an activin polypeptide, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (*see*, for example, Antibodies: A Laboratory Manual ed. by Harlow and Lane (Cold

Spring Harbor Press: 1988)). A mammal, such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the ActRII receptor polypeptide, an antigenic fragment which is capable of eliciting an antibody response, or a fusion protein. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of an ActRII receptor or activin polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

[00281] Following immunization of an animal with an antigenic preparation of an ActRII receptor polypeptide, antisera can be obtained and, if desired, polyclonal antibodies can be isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by Kohler and Milstein, (1975) Nature, 256: 495-497), the human B cell hybridoma technique (Kozbar *et al.*, (1983) Immunology Today, 4: 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, (1985) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with an ActRII receptor polypeptide and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

[00282] The term "antibody" as used herein is intended to include fragments thereof which are also specifically reactive with a subject polypeptide. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab)2 fragments can be generated by treating antibody with pepsin. The resulting F(ab)2 fragment can be treated to reduce disulfide bridges to produce Fab fragments. An antibody is further intended to include bispecific, single-chain, chimeric, humanized and fully human molecules having affinity for an ActRII receptor or activin polypeptide conferred by at least one CDR region of the antibody. An antibody may further comprise a label attached thereto and able to be detected (*e.g.*, the label can be a radioisotope, fluorescent compound, enzyme or enzyme co-factor).

[00283] In certain embodiments, the antibody is a recombinant antibody, which term encompasses any antibody generated in part by techniques of molecular biology, including CDR-

grafted or chimeric antibodies, human or other antibodies assembled from library-selected antibody domains, single chain antibodies and single domain antibodies (*e.g.*, human V_H proteins or camelid V_{HH} proteins). In certain embodiments, an antibody can be a monoclonal antibody, and in certain embodiments. For example, a method for generating a monoclonal antibody that binds specifically to an ActRII receptor polypeptide or activin polypeptide may comprise administering to a mouse an amount of an immunogenic composition comprising the antigen polypeptide effective to stimulate a detectable immune response, obtaining antibody-producing cells (*e.g.*, cells from the spleen) from the mouse and fusing the antibody-producing cells with myeloma cells to obtain antibody-producing hybridomas, and testing the antibody-producing hybridomas to identify a hybridoma that produces a monoclonal antibody that binds specifically to the antigen. Once obtained, a hybridoma can be propagated in a cell culture, optionally in culture conditions where the hybridoma-derived cells produce the monoclonal antibody that binds specifically to the antigen. The monoclonal antibody may be purified from the cell culture.

[00284] The adjective "specifically reactive with" as used in reference to an antibody is intended to mean, as is generally understood in the art, that the antibody is sufficiently selective between the antigen of interest (*e.g.*, an ActRII receptor polypeptide) and other antigens that are not of interest that the antibody is useful for, at minimum, detecting the presence of the antigen of interest in a particular type of biological sample. In certain methods employing the antibody, such as therapeutic applications, a higher degree of specificity in binding may be desirable. Monoclonal antibodies generally have a greater tendency (as compared to polyclonal antibodies) to discriminate effectively between the desired antigens and cross-reacting polypeptides. One characteristic that influences the specificity of an antibody:antigen interaction is the affinity of the antibody for the antigen. Although the desired specificity may be reached with a range of different affinities, generally preferred antibodies will have an affinity (a dissociation constant) of about 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} or less. Given the extraordinarily tight binding between activin and an ActRII receptor, it is expected that a neutralizing anti-activin or anti-ActRII receptor antibody would generally have a dissociation constant of 10^{-10} or less.

[00285] In addition, the techniques used to screen antibodies in order to identify a desirable antibody may influence the properties of the antibody obtained. For example, if an antibody is to be used for binding an antigen in solution, it may be desirable to test solution binding. A variety of different techniques are available for testing interaction between antibodies and antigens to identify particularly desirable antibodies. Such techniques include ELISAs,

surface plasmon resonance binding assays (*e.g.*, the Biacore.TM. binding assay, Biacore AB, Uppsala, Sweden), sandwich assays (*e.g.*, the paramagnetic bead system of IGEN International, Inc., Gaithersburg, Md.), Western blots, immunoprecipitation assays, and immunohistochemistry.

[00286] In certain embodiments, ActRII signaling inhibitors to be used in the compositions and methods described herein include alternative forms of activin, particularly those with alterations in the type I receptor binding domain can bind to type II receptors and fail to form an active ternary complex. In certain embodiments, nucleic acids, such as antisense molecules, siRNAs or ribozymes that inhibit activin A, B, C or E, or, particularly, ActRII receptor expression, can be used in the compositions and methods described herein. In certain embodiments, the ActRII signaling inhibitors to be used in the compositions and methods described herein exhibit selectivity for inhibiting GDF11-mediated signaling versus other members of the TGF-beta family, particularly with respect to GDF8 and activin.

[00287] In other embodiments, the inhibitors of ActRII signaling used in the compositions and methods described herein are non-antibody proteins with ActRII receptor antagonist activity, including inhibin (*i.e.*, inhibin alpha subunit), follistatin (*e.g.*, follistatin-288 and follistatin-315), Cerberus, follistatin related protein ("FSRP"), endoglin, activin C, alpha(2)-macroglobulin, and an M108A (methionine to alanine change at position 108) mutant activin A.

[00288] In a specific embodiment, the ActRII signaling inhibitor to be used in the compositions and methods described herein is a follistatin polypeptide that antagonizes activin bioactivity and/or binds to activin. The term "follistatin polypeptide" includes polypeptides comprising any naturally occurring polypeptide of follistatin as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity, and further includes any functional monomer or multimer of follistatin. Variants of follistatin polypeptides that retain activin binding properties can be identified based on previous studies involving follistatin and activin interactions. For example, WO2008/030367, which is included by reference herein in its entirety, discloses specific follistatin domains ("FSDs") that are shown to be important for activin binding. Follistatin polypeptides include polypeptides derived from the sequence of any known follistatin having a sequence at least about 80% identical to the sequence of a follistatin polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. Examples of follistatin polypeptides include the mature follistatin polypeptide or shorter isoforms or other variants of the human follistatin precursor polypeptide

as described, for example, in WO2005/025601, which is included by reference herein in its entirety.

[00289] In a specific embodiment, the ActRII signaling inhibitor to be used in the compositions and methods described herein is a follistatin-like related gene (FLRG) that antagonizes activin bioactivity and/or binds to activin. The term "FLRG polypeptide" includes polypeptides comprising any naturally occurring polypeptide of FLRG as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Variants of FLRG polypeptides that retain activin binding properties can be identified using routine methods to assay FLRG and activin interactions. *See*, for example, U.S. Pat. No. 6,537,966, which is included by reference herein in its entirety. FLRG polypeptides include polypeptides derived from the sequence of any known FLRG having a sequence at least about 80% identical to the sequence of an FLRG polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity.

[00290] In certain embodiments, functional variants or modified forms of the follistatin polypeptides and FLRG polypeptides include fusion proteins having at least a portion of the follistatin polypeptides or FLRG polypeptides and one or more fusion domains, such as, for example, domains that facilitate isolation, detection, stabilization or multimerization of the polypeptide. Suitable fusion domains are discussed in detail above with reference to the ActRIIA and ActRIIB polypeptides. In one embodiment, an ActRII signaling inhibitor is a fusion protein comprising an activin binding portion of a follistatin polypeptide fused to an Fc domain. In another embodiment, an ActRII signaling inhibitor is a fusion protein comprising an activin binding portion of an FLRG polypeptide fused to an Fc domain.

8.6 ASSAYS

[00291] Various ActRII polypeptide variants, or soluble ActRII polypeptide variants, may be tested for their ability to inhibit ActRII. In addition, compounds can be tested for their ability to inhibit ActRII. Once inhibitors of ActRII signaling activity are confirmed, these compounds can be used with the methods provided herein. ActRII can be ActRIIA or ActRIIB. The assays below are described for ActRIIA but can be performed analogously for ActRIIB.

8.6.1 ASSESSING SNAI1, PHOSPHOSMAD2, PHOSPHOSMAD3, URINARY PROTEIN, DKK1, COL1A1, ACTIVIN (*E.G.*, FREE ACTIVIN), RUNX2, ALP, BSAP, CTX, OSTERIX, KLOTHO, ALPHA-SMA, MYOCD, ACTRIIA, AXIN2, AND/OR SM22-ALPHA LEVEL AND/OR ACTIVITY

[00292] The level and/or the activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha can be determined by any method known in the art or described herein. For example, the level of Snai1, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample can be determined by assessing (*e.g.*, quantifying) transcribed RNA of Snai1, urinary protein, Dkk1, col1a1, activin, Runx2, Osterix, Alp, BSAP, CTX, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the sample using, *e.g.*, Northern blotting, PCR analysis, real time PCR analysis, or any other technique known in the art or described herein. It will be recognized by one of skill in the art, levels and/or activity of one or more of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, Sm22-alpha, phosphosmad3, and urinary protein can be compared independently and respectively to the level and/or activity in a corresponding reference population, such as described above in Section 8.3.1. In one embodiment, the level of Snai1, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample can be determined by assessing (*e.g.*, quantifying) mRNA of Snai1, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the sample. In a specific embodiment, the mRNA level of Snai1, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). In a specific embodiment, the nucleic acid sequence of the Runx2 mRNA comprises the nucleic acid sequence of SEQ ID NO: 58. In a specific embodiment, the nucleic acid sequence of the Alp mRNA comprises the nucleic acid sequence of SEQ ID NO: 59. In a specific embodiment, the nucleic acid sequence of the Snai1 mRNA comprises the nucleic acid sequence of SEQ ID NO: 74. In a specific embodiment, the nucleic acid sequence of the Dkk1 mRNA comprises the nucleic acid sequence of SEQ ID NO: 75. In a specific embodiment, the nucleic acid sequence of the col1a1 mRNA comprises the nucleic acid sequence of SEQ ID NO: 76. In a specific

embodiment, the nucleic acid sequence of the activin mRNA comprises the nucleic acid sequence of SEQ ID NO: 77. In a specific embodiment, the nucleic acid sequence of the Osterix mRNA comprises the nucleic acid sequence of SEQ ID NO: 60. In a specific embodiment, the nucleic acid sequence of the Klotho mRNA comprises the nucleic acid sequence of SEQ ID NO: 61. In a specific embodiment, the nucleic acid sequence of the alpha-SMA mRNA comprises the nucleic acid sequence of SEQ ID NO: 70. In a specific embodiment, the nucleic acid sequence of the MYOCD mRNA comprises the nucleic acid sequence of SEQ ID NO: 71. In a specific embodiment, the nucleic acid sequence of the Sm22-alpha mRNA comprises the nucleic acid sequence of SEQ ID NO: 62. In a specific embodiment, the qRT-PCR is performed with Runx2-specific primers (SEQ ID NOS: 48 and 49) to determine Runx2 levels. In a specific embodiment, the qRT-PCR is performed with Alp-specific primers (SEQ ID NOS: 50 and 51) to determine Alp levels. In a specific embodiment, the qRT-PCR is performed with Snai1-specific primers (SEQ ID NOS: 78 and 79) to determine Snai1 levels. In a specific embodiment, the qRT-PCR is performed with Dkk1-specific primers (SEQ ID NOS: 80 and 81) to determine Dkk1 levels. In a specific embodiment, the qRT-PCR is performed with col1a1-specific primers (SEQ ID NOS: 82 and 83) to determine col1a1 levels. In a specific embodiment, the qRT-PCR is performed with activin-specific primers (SEQ ID NOS: 84 and 85) to determine activin levels. In a specific embodiment, the qRT-PCR is performed with Osterix-specific primers (SEQ ID NOS: 52 and 53) to determine Osterix levels. In a specific embodiment, the qRT-PCR is performed with Klotho-specific primers (SEQ ID NOS: 54 and 55) to determine Klotho levels. In a specific embodiment, the qRT-PCR is performed with Sm22-alpha-specific primers (SEQ ID NOS: 56 and 57) to determine Sm22-alpha levels.

[00293] The level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample can also be determined by assessing (*e.g.*, quantifying) the level of protein expression of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the sample using, *e.g.*, immunohistochemical analysis, Western blotting, ELISA, immunoprecipitation, flow cytometry analysis, or any other technique known in the art or described herein. In a specific embodiment, the amino acid sequence of the Runx2 protein comprises the amino acid sequence of SEQ ID NO: 63. In a specific embodiment, the amino acid sequence of the Alp protein

comprises the amino acid sequence of SEQ ID NO: 64. In a specific embodiment, the amino acid sequence of the Snai1 protein comprises the amino acid sequence of SEQ ID NO: 86. In a specific embodiment, the amino acid sequence of the Dkk1 protein comprises the amino acid sequence of SEQ ID NO: 87. In a specific embodiment, the amino acid sequence of the col1a1 protein comprises the amino acid sequence of SEQ ID NO: 88. In a specific embodiment, the amino acid sequence of the activin (*e.g.*, free activin) protein comprises the amino acid sequence of SEQ ID NO: 89. In a specific embodiment, the amino acid sequence of the BSAP protein comprises the amino acid sequence of SEQ ID NO: 72. In a specific embodiment, the amino acid sequence of the Osterix protein comprises the amino acid sequence of SEQ ID NO: 65. In a specific embodiment, the amino acid sequence of the Klotho protein comprises the amino acid sequence of SEQ ID NO: 66. In a specific embodiment, the amino acid sequence of the alpha-SMA protein comprises the amino acid sequence of SEQ ID NO: 68. In a specific embodiment, the amino acid sequence of the MYOCD comprises the amino acid sequence of SEQ ID NO: 69. In a specific embodiment, the amino acid sequence of the Sm22-alpha protein comprises the amino acid sequence of SEQ ID NO: 67. In a specific embodiment, the amino acid sequence of the ActRIIA protein comprises the amino acid sequence of SEQ ID NO: 1. In a specific embodiment, the amino acid sequence of the ActRIIA protein comprises the amino acid sequence of SEQ ID NO: 2. In a specific embodiment, the amino acid sequence of the ActRIIA protein comprises the amino acid sequence of SEQ ID NO: 3. In a specific embodiment, the amino acid sequence of the ActRIIA protein comprises the amino acid sequence of SEQ ID NO: 4. In a specific embodiment, the amino acid sequence of the ActRIIA protein comprises the amino acid sequence of SEQ ID NO: 5. In a specific embodiment, the amino acid sequence of the Axin2 protein comprises the amino acid sequence of SEQ ID NO: 87. In particular embodiments, the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is determined by western blot. In a particular embodiment, the activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is determined by western blot analysis of a protein modulated by Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, or SM22-alpha. In particular embodiments, the level of Snai1, phosphosmad2,

phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is determined by a method capable of quantifying the amount of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, present in a tissue sample of a subject (*e.g.*, in human serum), and/or capable of detecting the correction of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, following treatment with an ActRII signaling inhibitor. In one embodiment, the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample is determined by assessing (*e.g.*, quantifying) protein expression of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the sample using ELISA. For example, Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha can be identified and quantified in the human serum using ELISA method. The ELISA method for use in determining the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample can comprise coating of ELISA plates with the tissue sample (*e.g.*, human serum), and detecting Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the tissue sample (*e.g.*, human serum) that bind to Snai1-, phosphosmad2-, phosphosmad3-, urinary protein-, Dkk1-, col1a1-, activin- (*e.g.*, free activin), Runx2-, Alp-, BSAP-, CTX-, Osterix-, Klotho-, alpha-SMA-, MYOCD-, ActRIIA-, Axin2-, and/or Sm22-alpha-specific antibodies, respectively. In some embodiments, the method for use in determining the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha as described herein (*e.g.*, ELISA) is capable of detecting from 100 pg/ml of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1,

col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha. In a specific embodiment, the ELISA is performed with Runx2-specific antibody SC-390715 (Santa Cruz) to determine Runx2 levels. In a specific embodiment, the ELISA is performed with Alp-specific antibody SC-98652 (Santa Cruz) to determine Alp levels. In a specific embodiment, the ELISA is performed with Snai1-specific antibody sc-393172 (Santa Cruz) to determine Snai1 levels. In a specific embodiment, the ELISA is performed with phosphosmad2-specific antibody sc-101801 (Santa Cruz) to determine phosphosmad2 levels. In a specific embodiment, the ELISA is performed with phosphosmad3-specific antibody sc-130218 (Santa Cruz) to determine phosphosmad2 levels. In a specific embodiment, the ELISA is performed with Dkk1-specific antibody sc-374574 (Santa Cruz) to determine Dkk1 levels. In a specific embodiment, the ELISA is performed with col1a1-specific antibody sc-8784 (Santa Cruz) to determine col1a1 levels. In a specific embodiment, the ELISA is performed with activin-specific antibody A1594 (Sigma Aldrich) to determine activin (*e.g.*, free activin) levels. In a specific embodiment, the ELISA is performed with BSAP-specific antibody SC-98652 (Santa Cruz) to determine CTX levels. In a specific embodiment, the ELISA is performed with CTX-specific antibody ABIN1173415 (Antibodies Online) to determine CTX levels. In a specific embodiment, the ELISA is performed with Osterix-specific antibody SC-22538 (Santa Cruz) to determine Osterix levels. In a specific embodiment, the ELISA is performed with Klotho-specific antibody SC-22218 (Santa Cruz) to determine Klotho levels. In a specific embodiment, the ELISA is performed with alpha-SMA-specific antibody SC-53142 (Santa Cruz) to determine alpha-SMA levels. In a specific embodiment, the ELISA is performed with MYOCD-specific antibody SC-21561 (Santa Cruz) to determine MYOCD levels. In a specific embodiment, the ELISA is performed with Sm22-alpha-specific antibody SC-271719 (Santa Cruz) to determine Sm22-alpha levels. In a specific embodiment, the ELISA is performed with ActRIIA-specific antibody ab 135634 (Abcam) to determine ActRIIA levels.

[00294] The levels of one or more of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, Sm22-alpha, phosphosmad3, and urinary protein described in the Elisa assays described herein can be compared independently and respectively to the level and/or activity in a corresponding reference population, such as described above in Section 8.3.1.

[00295] Antibodies for use in assays that measure the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP,

CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a sample (*e.g.*, in a tissue sample, *e.g.*, a sample of aorta, blood, serum, plasma, liver, spleen, and/or bone marrow) are known in the art or could be readily developed using approaches known to those of skill in the art. Examples of monoclonal antibodies that can be used in assays that measure the levels of Runx2 in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-B4293, LS-B4294, LS-B4296; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number sc-390715; and antibodies available from Sigma-Aldrich Co. LLC, with product number: WH0000860M1. Examples of monoclonal antibodies that can be used in assays that measure the levels of Alp in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-B2877, LS-B1844, LS-C169212; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: sc-98652; and antibodies available from Sigma-Aldrich Co. LLC, with product number: SAB1405449. Examples of monoclonal antibodies that can be used in assays that measure the levels of Snai1 in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-C161335, LS-C198229, and LS-C169298; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: sc-393172; and antibodies available from Sigma-Aldrich Co. LLC, with product number: SAB1404386 Examples of antibodies that can be used in assays that measure the levels of phosphosmad2 in a sample include antibodies from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: sc-101801; and antibodies available from Sigma-Aldrich Co. LLC, with product numbers: SAB4200251 and SAB4300252. Examples of antibodies that can be used in assays that measure the levels of phosphosmad3 in a sample include antibodies from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: sc-130218; and antibodies available from Sigma-Aldrich Co. LLC, with product numbers: SAB4300253 and SAB4504210. Examples of monoclonal antibodies that can be used in assays that measure the levels of Dkk1 in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-C108793, LS-C105116, and LS-C105117; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: sc-374574; and antibodies available from Sigma-Aldrich Co. LLC, with product number: WH0022943M1. Examples of monoclonal antibodies that can be used in assays that measure the levels of col1a1 in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog number LS-B5932; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number:

sc-8784; and antibodies available from Sigma-Aldrich Co. LLC, with product number: C2456. Examples of monoclonal antibodies that can be used in assays that measure the levels of activin (*e.g.*, free activin) in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog number LS-C308491; and antibodies available from Sigma-Aldrich Co. LLC, with product number: A1719. Examples of monoclonal antibodies that can be used in assays that measure the levels of BSAP in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-B2877, LS-B1844, LS-C169212; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: sc-98652; and antibodies available from Sigma-Aldrich Co. LLC, with product number: SAB1405449. Examples of monoclonal antibodies that can be used in assays that measure the levels of CTX in a sample include ABIN1173415 (Antibodies Online). Examples of monoclonal antibodies that can be used in assays that measure the levels of Osterix in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-C139215, LS-C132610, LS-B6531; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: sc-133871; and antibodies available from Sigma-Aldrich Co. LLC, with product number: WH0121340M1. Examples of monoclonal antibodies that can be used in assays that measure the levels of Klotho in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-C165587, LS-C145689, LS-C8376; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: sc-22218; and antibodies available from Sigma-Aldrich Co. LLC, with product number: SAB1306662. Examples of antibodies that can be used in assays that measure the levels of alpha-SMA in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-B6000, LS-B5966, LS-B2161; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: SC-21561; and antibodies available from Sigma-Aldrich Co. LLC, with product number: A5228. Examples of antibodies that can be used in assays that measure the levels of MYOCD in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-C37407, LS-C153495, LS-c137255; antibodies available from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, with catalog number: SC-53142; and antibodies available from Sigma-Aldrich Co. LLC, with product number: M8948. Examples of monoclonal antibodies that can be used in assays that measure the levels of Sm22-alpha in a sample include antibodies from LifeSpan Biosciences Inc., Seattle, WA, with catalog numbers LS-B2563, LS-C139114, LS-C210979; antibodies available from Santa Cruz Biotechnology,

Inc., Santa Cruz, CA, with catalog number: sc-271719; and antibodies available from Sigma-Aldrich Co. LLC, with product number: SAB2501014. Examples of monoclonal antibodies that can be used in assays that measure the levels of ActRIIA in a sample include antibodies from Abcam with product code: ab135634.

[00296] The activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha can be measured by any assay known in the art including, without limitation, a reporter gene assay (*e.g.*, containing Snai1-, phosphosmad2-, phosphosmad3-, urinary protein-, Dkk1-, col1a1-, activin-, Runx2-, Alp-, BSAP-, CTX-, Osterix-, Klotho-, alpha-SMA-, MYOCD-, ActRIIA-, Axin2-, or Sm22-alpha-responsive reporter gene construct, respectively) or any other bioactivity assay.

[00297] The level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha can be assessed in any tissue sample obtained from a subject treated in accordance with the methods described herein. In certain embodiments, Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha level and/or activity is assessed in a sample obtained from aorta, serum, liver, spleen or bone marrow of a subject treated in accordance with the methods described herein. In one embodiment, Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha level and/or activity is assessed in a sample obtained from serum of a subject treated in accordance with the methods described herein. In another embodiment, Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha level and/or activity is assessed in a sample obtained from aorta, spleen of a subject treated in accordance with the methods described herein. In yet another embodiment, Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha level and/or activity is assessed in a sample obtained from bone marrow of a subject treated in accordance with the methods described herein.

[00298] It will be recognized by one skilled in the art that the activity of one or more of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, Sm22-alpha, phosphosmad3, and urinary protein determined using assays known in the art and described herein, can be compared respectively to the level and/or activity in a corresponding reference population, such as described above in Section 8.3.1.

[00299] In some embodiments, the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample of a subject is compared to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in tissue samples (*e.g.*, in samples from the same tissue) of a reference population as described in Section 8.6. In some embodiments, the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample of a subject is compared to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a tissue sample (*e.g.*, in a sample from the same tissue) of the subject at an earlier time point (*e.g.*, before the onset of disease, before the onset of treatment, or during treatment). In some embodiments, the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample (*e.g.*, aorta, serum, spleen, liver, or blood marrow) of a subject is compared to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, and/or Sm22-alpha, respectively, in another tissue sample of the subject. In some embodiments, the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample of a subject is compared to the level and/or activity of another gene product in the tissue sample of the subject (*e.g.*, b-actin, Activin A, Activin B).

[00300] In some embodiments, the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample of a subject is compared to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the tissue sample in a reference population. In some embodiments, detection of the elevated level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in comparison to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population, is followed by administration of an activin receptor signaling inhibitor (such as one or more activin receptor signaling inhibitors described herein). In some embodiments, administration of an activin receptor signaling inhibitor (such as one or more activin receptor signaling inhibitors described herein) is followed by monitoring of the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha and, optionally, comparing the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population. In some embodiments, administration of a first dose of ActRII signaling inhibitor (*e.g.*, ActRIIA-hFc such as SEQ ID NO:7) is followed by determining the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, and if the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix is elevated as compared to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, respectively,

in a reference population, and/or if the level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is decreased as compared to the level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population, administering a second dose of the ActRII signaling inhibitor which is higher (*e.g.*, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, or 10 times higher) than the first dose, and if the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix is decreased as compared to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, respectively, in a reference population, and/or if the level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is elevated as compared to the level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population, administering a second dose of the ActRII signaling inhibitor which is lower (*e.g.*, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, or 10 times lower) than the first dose. In certain embodiments, the reference population is a population as described in Section 8.6.

[00301] In some embodiments, the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in a tissue sample of the treated subject is compared to the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a sample from the same tissue of one or more healthy subjects. In some embodiments, the tissue in which the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is assessed is aorta, blood serum, bone marrow, liver, or spleen. In one embodiment, the tissue in which the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is assessed is serum.

[00302] In certain embodiments, the elevated level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix is about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%,

80% 90%, 100%, 200%, or 500% greater than the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, respectively, in a reference population; and/or the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, or 100% less than the level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population. In certain embodiments, the elevated level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix is equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 200%, or 500% greater than the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, respectively, in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population; and/or the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population. In certain embodiments, the reference population is as described in Section 8.6.

[00303] In certain embodiments, the elevated level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, 100%, 200%, or 500% greater than the level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the reference population; and/or the decreased level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix is about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, or 100% less than the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix, respectively, in a reference population. In certain embodiments, the elevated level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 200%, or 500% greater than the level and/or activity of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population; and/or the

decreased level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix is equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, and/or Osterix in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population. In certain embodiments, the reference population is as described in Section 8.6.

[00304] In certain embodiments, the levels of activin are the levels of free activin , such as, for example, activin not in association with, for example, follistatin, follistatin-like 3, or inhibin. The level of free activin can be determined by, for example, (i) quantifying the concentration of activin in a sample, *e.g.*, in the plasma; (ii) quantifying the concentration of proteins that associate with activin, such as, *e.g.*, follistatin, follistatin-like 3, and inhibin in the sample; and (iii) calculating the stoichiometric ratio of the activin concentration to the concentration of the activin-associated proteins.

[00305] The assays described herein can also be utilized to determine the level and/or activity of other proteins and/or transcripts, such as, for example, FGF23, follistatin, follistatin-like 3, inhibin, proteins and/or transcripts involved in endothelial to mesenchymal transition.

[00306] In certain embodiments, the level and/or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is in a tissue (*e.g.*, tissue sample). In certain embodiments, the tissue is aorta. In certain embodiments, the tissue is kidney. In certain embodiments, the tissue is bone. In certain embodiments, the tissue is serum. In a preferred embodiment, the Runx2, Dkk1, Alp, osterix, sm22-alpha, Klotho, alpha-SMA, ActRIIA, Axin2, and MYOCD levels and/or activity are in aorta. In a preferred embodiment, the phosphosmad2, phosphosmad3, urinary protein, ActRIIA, Axin2, and col1a1 levels and/or activity are in kidney. In a preferred embodiment, the activin levels and/or activity are in serum. In certain embodiments, the elevated activin levels are in peritubular myofibroblasts. In certain embodiments, the elevated activin levels are not in the renal epithelium. In certain embodiments, the elevated activin levels are in the peritubular myofibroblasts, and the elevated activin levels are not in the renal epithelium.

[00307] It will be recognized by one skilled in the art that the levels and/or activity of one or more of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix,

Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, Sm22-alpha, phosphosmad3, and urinary protein can be compared independently and respectively to the level and/or activity in a corresponding reference population, such as described above in Section 8.3.1.

8.6.2 REFERENCE POPULATION

[00308] In certain embodiments, data (*e.g.*, biomarker levels or clinical symptoms) obtained from a reference population described herein is utilized to determine whether analogous data obtained from a subject treated or to be treated in accordance with the methods provided herein is pathologically high (*e.g.*, increased) or low (*e.g.*, decreased).

[00309] In certain embodiments, the size of the reference population can be 1, 5, 10, 25, 50, 75, 100, 200, 250, 300, 400, 500, or 1000 individuals. In certain embodiments, the reference population consists of random volunteers. In certain embodiments, the reference population consists of healthy people. In certain embodiments, the reference population consists of people of the same age, weight, and/or gender as the patient population as described in Section 8.4. In certain embodiments, the reference population consists of people without cardiovascular disease. In certain embodiments, the reference population consists of people without vascular calcification. In certain embodiments, the reference population consists of people without cardiovascular disease. In certain embodiments, the reference population consists of people without cardiovascular disease associated with and/or resulting from vascular calcification. In certain embodiments, the reference population consists of people without renal disease. In certain embodiments, the reference population consists of people without chronic kidney disease. In certain embodiments, the reference population consists of people without pathologically elevated levels of arterial stiffness. In certain embodiments, the reference population consists of people without cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness. In certain embodiments, the reference population consists of people without LVH. In certain embodiments, the reference population consists of people without cardiovascular disease associated with and/or resulting from LVH.

[00310] In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to the onset of one or more symptoms of cardiovascular disease. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to the onset of one or more symptoms of vascular calcification. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to the onset of one or more

symptoms of chronic kidney disease. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to the onset of one or more symptoms of elevated levels of arterial stiffness. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to the onset of one or more symptoms of LVH. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of cardiovascular disease in the subject. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of vascular calcification in the subject. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of chronic kidney disease in the subject. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of stage 1 chronic kidney disease. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of stage 2 chronic kidney disease. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of stage 3 chronic kidney disease. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of stage 4 chronic kidney disease. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of stage 5 chronic kidney disease. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of elevated levels of arterial stiffness in the subject. In certain embodiments, the reference population refers to a subject treated in accordance with the methods provided herein prior to diagnosis of LVH in the subject. In certain embodiments, the reference population consists of people who display an increase in arterial stiffness prior to being treated according to the methods provided herein or being diagnosed with a disease described herein. In certain embodiments, the reference population consists of people who display an upward trend of one of the markers recited in the Summary (*see* Section 6) prior to treatment according to the methods provided herein or being diagnosed with a disease described herein. In certain embodiments, the reference population consists of people who display an increased level of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, Osterix, phosphosmad3, and/or urinary protein prior to treatment according

to the methods provided herein or being diagnosed with a disease described herein. In certain embodiments, the reference population consists of people who display a downward trend of one of the markers recited in the Summary (*see* Section 6) prior to treatment according to the methods provided herein or being diagnosed with a disease described herein. In certain embodiments, the reference population consists of people who display a decreased level of alpha-SMA, MYOCD, Sm22-alpha, or ActRIIA prior to treatment according to the methods provided herein or being diagnosed with a disease described herein.

8.6.3 ENDOTHELIAL-MESENCHYMAL TRANSITION

[00311] In certain embodiments, EnMT in a subject treated according to the methods provided herein can be monitored through a lineage tracing assay to determine the fate of a cell population. In certain embodiments, EnMT in a subject treated according to the methods provided herein can be monitored by quantifying the level and/or activity of one or more proteins or transcripts associated with EnMT, such as, for example, Snai1.

8.6.4 BONE TURNOVER

[00312] Various circulating markers of bone turnover can be used to diagnose bone disorders, such as low bone turnover. Circulating markers of bone turnover are markers of bone formation such as bone specific alkaline phosphatase (bAP), osteocalcin, procollagen type I C-terminal propeptide (PICP) and insulin-like growth factor-1 (IGF-1), some being markers of bone resorption such as pyridinoline, deoxypyridinoline, tartrate-resistant acid phosphatase (TRAP), TRAP type 5b, pyridinoline, deoxypyridinoline and procollagen type I C-terminal telopeptide (ICTP), serum or urine collagen cross-links (N-telopeptide or C-telopeptide), and 25 hydroxyvitamin D. Assays to measure the entire parathyroid hormone (PTH) molecule can also be used. The skilled artisan is aware of imaging methods allowing the assessment of bone mineral density (BMD), bone volume, trabecular bone volume, and trabecular thickness. *See, e.g.*, Tilman B. Drueke and Sharon M. Moe, Disturbances of bone and mineral metabolism in chronic kidney disease: an international initiative to improve diagnosis and treatment, Nephrol Dial Transplant (2004) 19: 534–536; Okuno S, Inaba M., Biochemical markers of bone turnover. New aspect. Dialysis and bone metabolic marker, Clin Calcium. 2009 Aug;19(8):1084-91; Herberth J, Monier-Faugere MC, Mawad HW, Branscum AJ, Herberth Z, Wang G, Cantor T, Malluche HH, The five most commonly used intact parathyroid hormone assays are useful for screening but not for diagnosing bone turnover abnormalities in CKD-5 subjects, Clin Nephrol.

2009 Jul;72(1):5-14; Lehmann G, Ott U, Kaemmerer D, Schuetze J, Wolf G., Bone histomorphometry and biochemical markers of bone turnover in subjects with chronic kidney disease Stages 3 – 5, Clin Nephrol. 2008 Oct;70(4):296-305; Drücke TB., Is parathyroid hormone measurement useful for the diagnosis of renal bone disease?, Kidney Int. 2008 Mar;73(6):674-6; Yamada S, Inaba M, Kurajoh M, Shidara K, Imanishi Y, Ishimura E, Nishizawa Y., Utility of serum tartrate-resistant acid phosphatase (TRACP5b) as a bone resorption marker in subjects with chronic kidney disease: independence from renal dysfunction., Clin Endocrinol (Oxf). 2008 Aug;69(2):189-96. Epub 2008 Jan 23. *See* also, Paul D. Miller, Diagnosis and Treatment of Osteoporosis in Chronic Renal Disease, 2009.

[00313] Another marker for monitoring bone resorption in CKD subjects with mild renal dysfunction is serum concentration of type I collagen N-telopeptide (S-NTX). *See, e.g.*, Hamano T, Fujii N, Nagasawa Y, Isaka Y, Moriyama T, Okada N, Imai E, Horio M, Ito T., Serum NTX is a practical marker for assessing antiresorptive therapy for glucocorticoid treated subjects with chronic kidney disease., Bone. 2006 Nov;39(5):1067-72. Epub 2006 Jun 16.

[00314] Quantitative computed tomography (QCT) can also be used to determine bone turnover.

[00315] Markers, such as, for example, Runx2 and Alp can be evaluated to monitor the osteoblastic transition in a subject. Markers, such as, for example, Sm22-alpha can be evaluated to monitor vascular smooth muscle function and the levels of differentiated vascular smooth muscle cells.

8.6.5 CALCIUM LEVELS

[00316] Calcium levels can be assayed by methods known to one skilled in the art, such as, *e.g.*, calcium ion selective electrode. In certain embodiments, total calcium levels are measured in serum, blood, aorta, or urine.

8.6.6 VASCULAR CALCIFICATION

[00317] Non-contrast computed tomography (CT) for imaging the extent of coronary artery calcification (CAC) and contrast CT for noninvasive coronary angiography (CTA) are developments generally used to diagnose obstructive coronary disease. Radionuclide stress testing, coronary artery calcium scanning, and noninvasive coronary angiography for diagnostic and prognostic cardiac assessment can also be used. *See*: Berman DS, Shaw LJ, Hachamovitch R, Friedman JD, Polk DM, Hayes SW, Thomson LE, Germano G, Wong ND, Kang X, Rozanski

A., Comparative use of radionuclide stress testing, coronary artery calcium scanning, and noninvasive coronary angiography for diagnostic and prognostic cardiac assessment, Semin Nucl Med. 2007 Jan;37(1):2-16.

[00318] Coronary calcium screening results from asymptomatic subjects can be used as a comparison. For example, calcium screening results obtained prior to the onset of kidney disease can be used as a comparison when vascular calcification is related to the kidney disease.

[00319] Possible methods of detecting and quantifying coronary artery calcification (CAC) include, but are not limited to, x-ray computed tomography and myocardial perfusion single photon emission computed tomography (SPECT). Moser KW, O'Keefe JH Jr, Bateman TM, McGhie IA., Coronary calcium screening in asymptomatic subjects as a guide to risk factor modification and stress myocardial perfusion imaging, J Nucl Cardiol. 2003 Nov-Dec;10(6):590-8. Multi-detector computed tomography (MDCT) also can be used to detect vascular calcification (*see, e.g.*, Burrill *et al.*, 2007, Postgrad. Med. J. 83(985):698-704).

[00320] Another diagnostic method for vascular calcification is fluorine 18 fluorodeoxyglucose (FDG) uptake in the thoracic aortic wall at combined positron emission tomography (PET)/computed tomography (CT). *See*: Tatsumi M, Cohade C, Nakamoto Y, Wahl RL., Fluorodeoxyglucose uptake in the aortic wall at PET/CT: possible finding for active atherosclerosis, Radiology. 2003 Dec;229(3):831-7. Epub 2003 Oct 30.

[00321] In even another embodiment, ultrafast CT can be used to detect the presence of atherosclerotic coronary disease. *See, e.g.*, Breen JF, Sheedy PF 2nd, Schwartz RS, Stanson AW, Kaufmann RB, Moll PP, Rumberger JA, Coronary artery calcification detected with ultrafast CT as an indication of coronary artery disease, Radiology. 1992 Nov;185(2):435-9.

[00322] Electron-beam computed tomography scanning can also be used to diagnose coronary artery disease. *See*: Schmermund A, Baumgart D, Sack S, Möhlenkamp S, Grönemeyer D, Seibel R, Erbel R., Assessment of coronary calcification by electron-beam computed tomography in symptomatic subjects with normal, abnormal or equivocal exercise stress test, Eur Heart J. 2000 Oct;21(20):1674-82.

[00323] Another test for vascular calcification regards the plaque composition in plexogenic and thromboembolic pulmonary hypertension. Chronic thromboembolic pulmonary hypertension is associated with atherosclerotic plaques with glycophorin-rich pultaceous cores, and plexogenic pulmonary hypertension with fibrous plaques. Thromboembolic material plays a critical role in the formation of pultaceous cores, of which erythrocyte membrane derived

glycophorin is a major component. Thereby, chronic thromboembolic and plexogenic pulmonary hypertension (primary and secondary (Eisenmenger syndrome)) are investigated. *See*: Arbustini E, Morbini P, D'Armini AM, Repetto A, Minzioni G, Piovella F, Viganó M, Tavazzi L, Plaque composition in plexogenic and thromboembolic pulmonary hypertension: the critical role of thrombotic material in pultaceous core formation, Heart. 2002 Aug;88(2):177-82.

[00324] Agatston scoring, a calcium scoring system based on density measurements of deposited calcium plaques, can be used to quantify vascular calcification. In this system, levels of vascular calcification can be measured by multi-detector computed tomography (MDCT) and attenuations in the rate of progression in the Agatston score can be assessed (*see, e.g.*, Sharma *et al.*, 2010, Vasc. Health Risk Manag. 6:603-611).

[00325] Further, vascular calcification can be assessed using the methods described in Adragao *et al.*, 2004, Nephrol. Dial. Transplant 19:1480-1488.

[00326] Another assay for use in quantifying vascular calcification in a subject is the lesion-specific calcium score, which comprises a method of calcium measurement that results from a CT test for coronary artery calcification. This method is described by, *e.g.*, Akram and Voros, 2008, Int. J. cardiovac. Imaging 14:743-749.

8.6.7 HEART SIZE AND CARDIAC HYPERTROPHY

[00327] Heart size and cardiac hypertrophy can be determined by any method known to the skilled artisan, such as, for example, magnetic resonance imaging, electrocardiography, echocardiography, and noncontrast-enhanced cardiac computed tomography.

8.6.8 ARTERIAL STIFFNESS

[00328] The levels of arterial stiffness can be determined by any method known to the skilled artisan, such as, for example, ultrasonic Doppler tests, magnetic resonance imaging including magnetic resonance arteriography, computerized tomography (CT) including CT angiography, and other forms of angiography known in the art.

8.6.9 KIDNEY DISEASE

[00329] Glomerular filtration rate, inulin clearance, hyperphosphatemia, and BUN levels can be determined by any method known to the skilled artisan to determine kidney disease. Renal fibrosis and/or glomerulosclerosis can be diagnosed and/or monitored by any method known to the skilled artisan, such as, for example, biopsy of kidney tissue and examination of the

tissue for scarring. Renal fibrosis and/or glomerulosclerosis can also be diagnosed and/or monitored by, for example, measuring the glomerular filtration rate and/or performing ultrasound of the kidney. *See* website of the National Kidney Foundation.

8.6.10 ANIMAL MODELS

[00330] Atherosclerotic *low density lipoprotein receptor*-deficient *(ldlr-/-)* males (C57Bl/6J background) can be purchased from Jackson Laboratories and fed high fat diet (42% calories from fat) (Teklad #) beginning at 12 weeks of age. The mice are obese, insulin resistant at 22 weeks of age, diabetic at 28 weeks of age and hypercholesterolemic.

[00331] A two-step procedure can be utilized to create chronic kidney disease as described previously (Davies, M.R., *et al.*, 2003. J Am Soc Nephrol 14:1559-1567; Davies, M.R., *et al.*, 2005. J Am Soc Nephrol 16:917-928.). Electrocautery can be applied to the right kidney through a 2 cm flank incision at 12 weeks post natal, followed by left total nephrectomy at 14 weeks of age. The intensity of the cautery is varied to produce moderate (CKD-3) renal injury that is confirmed by inulin clearances at age 20 weeks. A control group of mice, wild type C57Bl/6J mice, are fed a regular chow diet, which is a normal renal function and diet group used for normative control values. A second group is *ldlr-/-* mice that are fed a high fat diet and sham operated, which have normal renal function, and serve as the control group to determine the effect of kidney disease. A third group is *ldlr-/-* mice with GFR reduced equivalent to human CKD stage 3 fed high fat diet (CKD-3) with euthanasia at 22 weeks, the baseline vascular calcification group (CKD-3). The fourth group is *ldlr-/-* mice with CKD-3 receiving subcutaneous injections of vehicle twice a week beginning at 22 weeks until euthanasia at 28 weeks (CKD-3 V). The fifth group is *ldlr-/-* mice with CKD-3 receiving subcutaneous injections of mActRIIA-Fc (Celgene, Summit, NJ), 10 mg/kg twice a week beginning at 22 weeks until euthanasia at 28 weeks (CKD-3 mActRIIA-Fc). The dose used was previously shown in PK/PD studies to be an efficacious dose for stimulation of bone formation (Lotinun, S., *et al.*, 2010. Bone 46:1082-1088.).

[00332] A second model of CKD used is the murine homolog of X-linked Alport's syndrome, which is a deficiency in the gene for the α5 chain of type IV collagen, COL4A5 (Rheault, M.N., *et al.*, 2004. Journal of the American Society of Nephrology 15:1466-1474.). This is a model of spontaneous kidney disease. Breeding pairs can be purchased from Jackson Laboratories and bred for experiments. Hemizygote males spontaneously develop kidney disease comparable with human CKD stage 3-4 at 200 days after birth.

[00333] A third model of CKD is renal ablation, similar to the *ldlr-/-* protocol, in a transgenic mouse line used for cellular lineage tracing, the GNZ mouse. GNZ reporter female mice (Stoller, J.Z., *et al.*, 2008. Genesis (New York, N.Y. 2000) 46:200-204.) and Tek-Cre transgenic male mouse (Koni, P.A., *et al.*, 2001. The Journal of Experimental Medicine 193:741-754.) can be purchased from Jackson Laboratories and bred to produce GNZ / Tek-Cre + mice for experiments. GNZ/ Tek-Cre – littermates serve as negative controls. Mouse genotyping can be performed by using specific primers recommended for GNZ and Tek-Cre mouse strains by manufacturer.

8.6.11 TRANSCRIPTIONAL RESPONSE ASSAY

[00334] In certain embodiments, a transcription response assay can be used to test an ActRII signaling inhibitor or activity of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Alp, BSAP, CTX, Osterix, Klotho, ActRIIA, Axin2, and/or Sm22-alpha. Upon ActRII, Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Osterix, Alp, BSAP, CTX, Klotho, ActRIIA, Axin2, and/or Sm22-alpha signaling, transcription of certain genes is up- or downregulated. A cell culture system used and the transcriptional response can be measured (*e.g.*, by RT-PCR). The effect of an agent on the transcriptional response is a measure of its effectiveness or activity. In certain embodiments, the promoter region that is known to be responsive to ActRII, Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free activin), Runx2, Osterix, Alp, BSAP, CTX, Klotho, ActRIIA, Axin2, and/or Sm22-alpha signaling can be cloned upstream of a reporter gene. In this way, the assay can be simplified such that only the activity of the reporter gene needs to be assayed.

8.6.12 SCREENING ASSAYS

[00335] Various ActRII polypeptide variants, or soluble ActRII polypeptide variants, may be tested for their ability to inhibit ActRII. In addition, compounds can be tested for their ability to inhibit ActRII. Once inhibitors of ActRII signaling activity are confirmed, these compounds can be used with the methods provided herein. ActRII can be ActRIIA or ActRIIB. The assays below are described for ActRIIA but can be performed analogously for ActRIIB.

[00336] For example, the effect of an ActRIIA polypeptide variant on the expression of genes involved in bone production or bone destruction may be assessed. This may, as needed, be performed in the presence of one or more recombinant ActRIIA ligand proteins (*e.g.*, activin),

and cells may be transfected so as to produce an ActRIIA polypeptide and/or variants thereof, and optionally, an ActRIIA ligand. Likewise, an ActRIIA polypeptide may be administered to a mouse or other animal, and one or more bone properties, such as density or volume may be assessed. The healing rate for bone fractures may also be evaluated. Dual-energy x-ray absorptiometry (DEXA) is a well-established, non-invasive, quantitative technique for assessing bone density in an animal. In humans central DEXA systems may be used to evaluate bone density in the spine and pelvis. These are the best predictors of overall bone density. Peripheral DEXA systems may be used to evaluate bone density in peripheral bones, including, for example, the bones of the hand, wrist, ankle and foot. Traditional x-ray imaging systems, including CAT scans, may be used to evaluate bone growth and fracture healing. In addition, bone density can be measured using quantitative computed tomography (qCT). The mechanical strength of bone may also be evaluated.

[00337] In certain aspects, provided herein is the use of ActRIIA polypeptides (*e.g.*, soluble ActRIIA polypeptides) and activin polypeptides to identify compounds (agents) which are agonist or antagonists of the activin-ActRIIA signaling pathway. Compounds identified through this screening can be tested to assess their ability to modulate bone growth or mineralization in vitro. Optionally, these compounds can further be tested in animal models to assess their ability to modulate tissue growth in vivo.

[00338] There are numerous approaches to screening for therapeutic agents for modulating tissue growth by targeting activin and ActRIIA polypeptides. In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb activin or ActRIIA-mediated effects on bone. In certain embodiments, the assay is carried out to screen and identify compounds that specifically inhibit or reduce binding of an ActRIIA polypeptide to activin. Alternatively, the assay can be used to identify compounds that enhance binding of an ActRIIA polypeptide to activin. In a further embodiment, the compounds can be identified by their ability to interact with an activin or ActRIIA polypeptide.

[00339] A variety of assay formats will suffice and, in light of the present disclosure, those not expressly described herein will nevertheless be comprehended by one of ordinary skill in the art. As described herein, the test compounds (agents) used herein may be created by any combinatorial chemical method. Alternatively, the subject compounds may be naturally occurring biomolecules synthesized in vivo or in vitro. Compounds (agents) to be tested for their ability to act as modulators of tissue growth can be produced, for example, by bacteria, yeast,

plants or other organisms (*e.g.*, natural products), produced chemically (*e.g.*, small molecules, including peptidomimetics), or produced recombinantly. Test compounds contemplated herein include non-peptidyl organic molecules, peptides, polypeptides, peptidomimetics, sugars, hormones, and nucleic acid molecules. In a specific embodiment, the test agent is a small organic molecule having a molecular weight of less than about 2,000 daltons.

[00340] The test compounds can be provided as single, discrete entities, or provided in libraries of greater complexity, such as made by combinatorial chemistry. These libraries can comprise, for example, alcohols, alkyl halides, amines, amides, esters, aldehydes, ethers and other classes of organic compounds. Presentation of test compounds to the test system can be in either an isolated form or as mixtures of compounds, especially in initial screening steps. Optionally, the compounds may be derivatized with other compounds and have derivatizing groups that facilitate isolation of the compounds. Non-limiting examples of derivatizing groups include biotin, fluorescein, digoxygenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S transferase (GST), photoactivatible crosslinkers or any combinations thereof.

[00341] In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity or bioavailability of the test compound can be generally ignored in the in vitro system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity between an ActRIIA polypeptide and activin.

[00342] Merely to illustrate, in an exemplary screening assay, the compound of interest is contacted with an isolated and purified ActRIIA polypeptide which is ordinarily capable of binding to activin. To the mixture of the compound and ActRIIA polypeptide is then added a composition containing an ActRIIA ligand. Detection and quantification of ActRIIA/activin complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the ActRIIA polypeptide and activin. The efficacy of the compound can be assessed by generating dose response curves from data obtained using

various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. For example, in a control assay, isolated and purified activin is added to a composition containing the ActRIIA polypeptide, and the formation of ActRIIA/activin complex is quantitated in the absence of the test compound. It will be understood that, in general, the order in which the reactants may be admixed can be varied, and can be admixed simultaneously. Moreover, in place of purified proteins, cellular extracts and lysates may be used to render a suitable cell-free assay system.

[00343] Complex formation between the ActRIIA polypeptide and activin may be detected by a variety of techniques. For instance, modulation of the formation of complexes can be quantitated using, for example, detectably labeled proteins such as radiolabeled (*e.g.*, ^{32}P, ^{35}S, ^{14}C or ^{3}H), fluorescently labeled (*e.g.*, FITC), or enzymatically labeled ActRIIA polypeptide or activin, by immunoassay, or by chromatographic detection.

[00344] In certain embodiments, contemplated herein is the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between an ActRIIA polypeptide and its binding protein. Further, other modes of detection, such as those based on optical waveguides (PCT Publication WO 96/26432 and U.S. Pat. No. 5,677,196), surface plasmon resonance (SPR), surface charge sensors, and surface force sensors, are compatible with many embodiments described herein.

[00345] Moreover, an interaction trap assay, also known as the "two hybrid assay," can be used for identifying agents that disrupt or potentiate interaction between an ActRIIA polypeptide and its binding protein. *See* for example, U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) Cell 72:223-232; Madura *et al.* (1993) J Biol Chem 268:12046-12054; Bartel *et al.* (1993) Biotechniques 14:920-924; and Iwabuchi *et al.* (1993) Oncogene 8:1693-1696). In a specific embodiment, contemplated herein is the use of reverse two hybrid systems to identify compounds (*e.g.*, small molecules or peptides) that dissociate interactions between an ActRIIA polypeptide and its binding protein. *See* for example, Vidal and Legrain, (1999) Nucleic Acids Res 27:919-29; Vidal and Legrain, (1999) Trends Biotechnol 17:374-81; and U.S. Pat. Nos. 5,525,490; 5,955,280; and 5,965,368.

[00346] In certain embodiments, the subject compounds are identified by their ability to interact with an ActRIIA or activin polypeptide. The interaction between the compound and the ActRIIA or activin polypeptide may be covalent or non-covalent. For example, such interaction

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

[00347] In certain aspects, provided herein are methods and agents for modulating (stimulating or inhibiting) bone formation and increasing bone mass. Therefore, any compound identified can be tested in whole cells or tissues, in vitro or in vivo, to confirm their ability to modulate bone growth or mineralization. Various methods known in the art can be utilized for this purpose. In particular, the compounds can be tested for their ability to increase bone turnover.

[00348] For example, the effect of the ActRIIA or activin polypeptides or test compounds on bone or cartilage growth can be determined by measuring induction of Msx2 or differentiation of osteoprogenitor cells into osteoblasts in cell based assays (*see*, *e.g.*, Daluiski *et al.*, Nat Genet. 2001, 27(1):84-8; Hino *et al.*, Front Biosci. 2004, 9:1520-9). Another example of cell-based assays includes analyzing the osteogenic activity of the subject ActRIIA or activin polypeptides and test compounds in mesenchymal progenitor and osteoblastic cells. To illustrate, recombinant adenoviruses expressing an activin or ActRIIA polypeptide can be constructed to infect pluripotent mesenchymal progenitor C3H10T1/2 cells, preosteoblastic C2Cl2 cells, and osteoblastic TE-85 cells. Osteogenic activity is then determined by measuring the induction of alkaline phosphatase, osteocalcin, and matrix mineralization (*see*, *e.g.*, Cheng *et al.*, J bone Joint Surg Am. 2003, 85-A(8): 1544-52).

[00349] Also provided herein are *in vivo* assays to measure bone or cartilage growth. For example, Namkung-Matthai *et al.*, Bone, 28:80-86 (2001) discloses a rat osteoporotic model in

which bone repair during the early period after fracture is studied. Kubo *et al.*, Steroid Biochemistry & Molecular Biology, 68:197-202 (1999) also discloses a rat osteoporotic model in which bone repair during the late period after fracture is studied. Andersson *et al.*, J. Endocrinol. 170:529-537 describe a mouse osteoporosis model in which mice are ovariectomized, which causes the mice to lose substantial bone mineral content and bone mineral density, with the trabecular bone losing roughly 50% of bone mineral density. Bone density could be increased in the ovariectomized mice by administration of factors such as parathyroid hormone. In certain aspects, fracture healing assays that are known in the art can be used. These assays include fracture technique, histological analysis, and biomechanical analysis, which are described in, for example, U.S. Pat. No. 6,521,750, which is incorporated by reference in its entirety for its disclosure of experimental protocols for causing as well as measuring the extent of fractures, and the repair process.

8.7 DOSE OF ACTIVIN RECEPTOR TYPE II SIGNALING INHIBITOR

[00350] In certain embodiments, an ActRII signaling inhibitor is an inhibitor of ActRIIA signaling as set forth in Section 8.5.1. In other embodiments, an ActRII inhibitor is an inhibitor of ActRIIB signaling as set forth in Section 8.5.2. In certain embodiments, an ActRII signaling inhibitor is a combination of an ActRIIA signaling inhibitor and an ActRIIB signaling inhibitor.

[00351] In certain embodiments, the ActRII signaling inhibitor is dosed at intervals and amounts sufficient to achieve serum concentrations of 0.2 microgram/kg or greater, and serum levels of 1 microgram/kg or 2 microgram/kg or greater are desirable for achieving significant effects on bone density and strength. Dosing regimens may be designed to reach serum concentrations of between 0.2 and 15 microgram/kg, and optionally between 1 and 5 microgram/kg. In humans, serum levels of 0.2 microgram/kg may be achieved with a single dose of 0.1 mg/kg or greater and serum levels of 1 microgram/kg may be achieved with a single dose of 0.3 mg/kg or greater. The observed serum half-life of the molecule is between about 20 and 30 days, substantially longer than most Fc fusion proteins, and thus a sustained effective serum level may be achieved, for example, by dosing with 0.2-0.4 mg/kg on a weekly or biweekly basis, or higher doses may be used with longer intervals between dosings. For example, doses of 1-3 mg/kg might be used on a monthly or bimonthly basis, and the effect on bone may be sufficiently durable that dosing is necessary only once every 3, 4, 5, 6, 9, 12 or more months. Serum levels of the ActRII signaling inhibitor can be measured by any means

known to the skilled artisan. For example, antibodies against the ActRII signaling inhibitor can be used to determine the serum levels of the ActRII signaling inhibitor using, *e.g.*, an ELISA.

[00352] In certain embodiments, the dose of the ActRII signaling inhibitor ranges from 0.01 to 3.0 mg/kg intravenously or from 0.03 to 0.1 mg/kg subcutaneously. In certain embodiments, the dose of ActRII signaling inhibitor is about 0.01 mg/kg, about 0.1 mg/kg, about 0.13 mg/kg, about 0.2 mg/kg, about 0.26 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.5 mg/kg, about 2.0 mg/kg, about 2.5 mg/kg, about 3.0 mg/kg, about 3.5 mg/kg, about 4.0 mg/kg, about 4.5 mg/kg, or about 5.0 mg/kg. In certain embodiments, the dose of ActRII signaling inhibitor is about 10.0 mg/kg, about 15.0 mg/kg, about 20.0 mg/kg, about 25.0 mg/kg, or about 30.0 mg/kg. In certain embodiments, the dose of ActRII signaling inhibitor is between 0.01 mg/kg and 0.1 mg/kg, between 0.1 mg/kg and 0.3 mg/kg, between 0.3 mg/kg and 0.5 mg/kg, between 0.3 mg/kg and 0.8 mg/kg, between 0.5 mg/kg and 1.0 mg/kg, between 1.0 mg/kg and 2.0 mg/kg, between 1.0 mg/kg and 3.0 mg/kg, between 2.0 mg/kg and 3.0 mg/kg, between 2.0 mg/kg and 4.0 mg/kg, between 3.0 mg/kg and 5.0 mg/kg, between 5.0 mg/kg and 10.0 mg/kg, between 10.0 mg/kg and 15.0 mg/kg, between 10.0 mg/kg and 20.0 mg/kg, between 15.0 mg/kg and 20.0 mg/kg, or between 20.0 mg/kg and 30.0 mg/kg. In certain embodiments, the dose is about 15 mg, about 30 mg, about 45 mg, about 60 mg, about 75 mg, about 90 mg, or about 1 g. In certain embodiments, the dose is about 0.1 mg/kg. In certain embodiments, the dose is about 0.3 mg/kg. In certain embodiments, the dose is about 0.5 mg/kg. In certain embodiments, the dose is about 0.7 mg/kg.

[00353] In certain embodiments, the dose is a pharmaceutically effective dose. In certain embodiments, the pharmaceutically effective dose is about 15 mg, about 30 mg, about 45 mg, about 60 mg, about 75 mg, about 90 mg, or about 1 g, or about 0.1 mg/kg, about 0.13 mg/kg, about 0.2 mg/kg, about 0.26 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg. In certain embodiments, the pharmaceutically effective dose is about 0.1 mg/kg. In certain embodiments, the pharmaceutically effective dose is about 0.3 mg/kg. In certain embodiments, the pharmaceutically effective dose is about 0.5 mg/kg. In certain embodiments, the pharmaceutically effective dose is about 0.7 mg/kg.

[00354] In certain embodiments, the dose is an initial dose. In certain embodiments, the initial dose is about 15 mg, about 30 mg, about 45 mg, about 60 mg, about 75 mg, about 90 mg, or about 1 g, or about 0.1 mg/kg, about 0.13 mg/kg, about 0.2 mg/kg, about 0.26 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg. In certain embodiments, the pharmaceutically effective dose is about 0.1 mg/kg. In certain embodiments, the initial dose is about 0.3 mg/kg. In certain embodiments, the initial dose is about 0.5 mg/kg. In certain embodiments, the initial dose is about 0.7 mg/kg. In certain embodiments, the initial dose is administered (i) once every 28 days; or (ii) once every 42 days. In certain embodiments, the initial dose is administered once every 14 days. In certain embodiments, the initial dose is administered once every 21 days.

[00355] In certain embodiments the initial dose is administered once every 1, 2, 3, 4, 5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days. In certain embodiments the initial dose is administered once every 1, 2, 3, 4, 5, or 6 weeks. In certain embodiments, the initial dose is administered once every 2 weeks. In certain embodiments, the initial dose is about 0.1 mg/kg, about 0.13 mg/kg, about 0.2 mg/kg, about 0.26 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg and is administered once every 1, 2, 3, 4, 5, or 6 weeks. In certain embodiments, the initial dose is between about 0.3 to about 0.8 mg/kg and is administered once every 2 weeks. In certain embodiments, the initial dose is about 0.1 mg/kg, about 0.13 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg and is administered once every 1, 2, 3, 4, 5, or 6 weeks. In certain embodiments, the initial dose is about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg and is administered once every 2 weeks. In certain embodiments, the initial dose is about 0.3 mg/kg and is administered once every 2 weeks. In certain embodiments, the initial dose is about 0.5 mg/kg and is administered once every 2 weeks. In certain embodiments, the initial dose is about 0.7 mg/kg and is administered once every 2 weeks. In certain embodiments, the initial dose is about 0.8 mg/kg and is administered once every 2 weeks.

[00356] In certain embodiments, the dose is an adjusted dose. In certain embodiments, the adjusted dose is greater than the initial dose. In certain embodiments, the adjusted dose is about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, or about 35 mg greater than the initial dose, or about 0.05 mg/kg, about 0.1 mg/kg, about 0.15 mg/kg, about 0.25 mg/kg, about 0.3 mg/kg, about 0.35 mg/kg, about 0.4 mg/kg, or about 0.5 mg/kg greater than the initial dose. In certain embodiments, the adjusted dose is administered more frequently than the initial dose. In certain embodiments, the adjusted dose is administered every 5, 10, 15, 20, 25, 28, 30, 35, or 40 days.

[00357] In certain embodiments, the adjusted dose is less than the initial dose. In certain embodiments, the adjusted dose is about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, or about 35 mg less than the initial dose, or about 0.05 mg/kg, about 0.1 mg/kg, about 0.15 mg/kg, about 0.25 mg/kg, about 0.3 mg/kg, about 0.35 mg/kg, about 0.4 mg/kg, or about 0.5 mg/kg less than the initial dose. In certain embodiments, the adjusted dose is administered less frequently than the initial dose. In certain embodiments, the adjusted dose is administered every 30, 35, 40, 42, 50, 60, 70, 80, or 90 days.

[00358] In certain embodiments, the dose is administered via injection. In certain embodiments, the dose is administered once every 28 days or once every 42 days. In certain embodiments, the dose is administered continuously and/or indefinitely.

[00359] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease activin levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to activin levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, the activin is free activin, *e.g.*, activin not associated with follistatin, follistatin-like 3, or inhibin. In certain embodiments, the activin is activin A. In certain embodiments, activin levels and/or activity are determined by an assay as described in Section 8.6.

[00360] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease Smad-dependent signaling in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%,

20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to Smad-dependent signaling in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Smad-dependent signaling is determined by an assay as described in Section 8.6.

[00361] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease Runx2 levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to Runx2 levels and/or activity of in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Runx2 levels and/or activity are determined by an assay as described in Section 8.6.

[00362] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease Alp levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to Alp levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Alp levels and/or activity are determined by an assay as described in Section 8.6.

[00363] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease Snai1 levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to Snai1 levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Snai1 levels and/or activity is determined by an assay as described in Section 8.6.

[00364] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease phosphosmad2 levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%,

15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to phosphosmad2 levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, phosphosmad2 levels and/or activity are determined by an assay as described in Section 8.6.

[00365] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease phosphosmad3 levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to phosphosmad3 levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, phosphosmad3 levels and/or activity are determined by an assay as described in Section 8.6.

[00366] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease urinary protein levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to urinary protein levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, urinary protein levels and/or activity are determined by an assay as described in Section 8.6.

[00367] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease Dkk1 levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to Dkk1 levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Dkk1 levels and/or activity are determined by an assay as described in Section 8.6.

[00368] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease col1a1 levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%,

20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to col1a1 levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, col1a1 levels and/or activity are determined by an assay as described in Section 8.6.

[00369] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease BSAP levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to BSAP levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, BSAP levels and/or activity are determined by an assay as described in Section 8.6.

[00370] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease CTX levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to CTX levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, CTX levels and/or activity are determined by an assay as described in Section 8.6.

[00371] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease Osterix levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to Osterix levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Osterix levels and/or activity are determined by an assay as described in Section 8.6.

[00372] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease ActRIIA levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%,

15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to ActRIIA levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, ActRIIA levels and/or activity are determined by an assay as described in Section 8.6.

[00373] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase Klotho levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to Klotho levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Klotho levels and/or activity are determined by an assay as described in Section 8.6.

[00374] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase alpha-SMA levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to alpha-SMA levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, alpha-SMA levels and/or activity are determined by an assay as described in Section 8.6.

[00375] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase MYOCD levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to MYOCD levels and/or activity in a reference population (*e.g.*, a reference population as described in

Section 8.6). In certain embodiments, MYOCD levels and/or activity are determined by an assay as described in Section 8.6.

[00376] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase Axin2 levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to Axin2 levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Axin2 levels and/or activity are determined by an assay as described in Section 8.6.

[00377] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase vascular smooth muscle protein levels, such as, for example, Sm22-alpha, in the subject as compared to vascular smooth muscle protein levels reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, vascular smooth muscle protein levels and/or activity are determined by an assay as described in Section 8.6. In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase Sm22-alpha levels and/or activity in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to Sm22-alpha levels and/or activity in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, Sm22-alpha levels and/or activity are determined by an assay as described in Section 8.6.

[00378] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase bone volume in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most

100% as compared to bone volume in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, bone volume is determined by an assay as described in Section 8.6.

[00379] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease osteoclast pit surface in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to osteoclast pit surface in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, osteoclast pit surface is determined by an assay as described in Section 8.6.

[00380] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to maintain bone formation rates in the subject, or minimally increase or decrease bone formation rates in the subject, such as, by at most 1%, 2.5%, 5%, 10%, or 15% as compared to bone formation rates in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, bone formation rate is determined by an assay as described in Section 8.6.

[00381] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to maintain the osteoblast surface in the subject, or minimally increase or decrease the osteoblast surface in the subject, such as, by at most 1%, 2.5%, 5%, 10%, or 15 as compared to osteoblast surface in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, osteoblast surface is determined by an assay as described in Section 8.6.

[00382] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease aortic osteoblastic transition in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to aortic osteoblastic transition in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, aortic osteoblast transition is determined by an assay as described in Section 8.6.

[00383] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease osteoblast number in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared osteoblast number in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, osteoblast number is determined by an assay as described in Section 8.6.

[00384] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease osteoblast surface to bone surface ratio in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared osteoblast surface to bone surface ratio in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, osteoblast surface to bone surface ratio is determined by an assay as described in Section 8.6.

[00385] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease osteoclast number in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared osteoclast number in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, osteoclast number is determined by an assay as described in Section 8.6.

[00386] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease osteoclast surface to bone surface ratio in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared osteoclast surface to bone surface ratio in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, osteoclast surface to bone surface ratio is determined by an assay as described in Section 8.6.

[00387] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase trabecular bone volume in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to trabecular bone volume in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, trabecular bone volume is determined by an assay as described in Section 8.6.

[00388] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase trabecular thickness in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to trabecular thickness in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, trabecular thickness is determined by an assay as described in Section 8.6.

[00389] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase vascular smooth muscle function in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to vascular smooth muscle function in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, vascular smooth muscle function is determined by an assay as described in Section 8.6.

[00390] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease vascular calcification in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%,

65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to vascular calcification in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, vascular calcification is determined by an assay as described in Section 8.6. In certain embodiments, the dose of an ActRII signaling inhibitor administered according to the methods provided herein is sufficient to decrease vascular calcium levels by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to vascular calcium levels in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, vascular calcium levels are determined by an assay as described in Section 8.6.

[00391] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease aortic calcium levels in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to aortic calcium levels in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, aortic calcium levels are determined by an assay as described in Section 8.6. In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease calcium deposits in aortic atheromas in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to calcium deposits in aortic atheromas in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, calcium deposits in aortic atheromas are determined by an assay as described in Section 8.6.

[00392] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease CKD-induced endothelial to mesenchymal transition (EnMT) in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least

100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to EnMT in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, EnMT is determined by an assay as described in Section 8.6.

[00393] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease heart size (*e.g.*, heart weight) in the subject by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or by at least 10%, or by at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or by at least 10% as compared to heart size (*e.g.*, heart weight) in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, heart size is determined by an assay as described in Section 8.6.

[00394] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase the levels of differentiated vascular smooth muscle cells in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at least 500%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or by at most 500% as compared to differentiated vascular smooth muscle cell levels in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, levels of differentiated vascular smooth muscle cells are determined by an assay as described in Section 8.6.

[00395] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to decrease elevated levels of arterial stiffness in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to arterial stiffness in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, arterial stiffness is determined by an assay as described in Section 8.6. In certain embodiments, the dose of an ActRII signaling inhibitor administered according to the methods provided herein is sufficient to decrease elevated levels of arterial stiffness in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least

100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to arterial stiffness in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, arterial stiffness is determined by an assay as described in Section 8.6.

[00396] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to maintain mineral apposition rate in the subject, or minimally increase or decrease minimal apposition rate in the subject, such as, by at most 1%, 2.5%, 5%, 10%, or 15% as compared to mineral apposition rate in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, mineral apposition rate is determined by an assay as described in Section 8.6.

[00397] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to maintain hyperphosphatemia in the subject, or minimally increase or decrease hyperphosphatemia in the subject, such as, by at most 1%, 2.5%, 5%, 10%, or 15% as compared to hyperphosphatemia in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, hyperphosphatemia is determined by an assay as described in Section 8.6.

[00398] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to maintain the levels of FGF23 in the subject, or minimally increase or decrease the levels of FGF23 in the subject, such as, by at most 1%, 2.5%, 5%, 10%, or 15% as compared to FGF23 levels in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, FGF levels are determined by an assay as described in Section 8.6.

[00399] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to reduce renal fibrosis in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to renal fibrosis in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, renal fibrosis is determined by an assay as described in Section 8.6. In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to reduce glomerulosclerosis in the subject by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%,

50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at least 100%, or by at most 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or at most 100% as compared to glomerulosclerosis in a reference population (*e.g.*, a reference population as described in Section 8.6). In certain embodiments, glomerulosclerosis is determined by an assay as described in Section 8.6.

[00400] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to normalize the levels of one or more of the biomarkers provided herein (*e.g.*, Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, urinary protein, and/or ActRIIA). For example, in certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to increase or decrease the levels of one or more of the biomarkers provided herein (*e.g.*, Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, urinary protein, and/or ActRIIA) to the levels of the respective biomarkers in a reference population (*e.g.*, a reference population as described in Section 8.6).

[00401] In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to treat and/or prevent cardiac hypertrophy in the subject. In certain embodiments, cardiac hypertrophy is determined by an assay as described in Section 8.6. In certain embodiments, the dose of an ActRII signaling inhibitor administered to a subject according to the methods provided herein is sufficient to treat and/or prevent LVH in the subject.

[00402] When used in conjunction with a dose provided herein (*e.g.*, a dose of an ActRII signaling inhibitor or a dose of a second active agent), the word "about" refers to any number within 1, 5 or 10% of the referenced number.

[00403] In certain embodiments, an ActRII signaling inhibitor as described herein is administered to a subject according to the methods provided herein subcutaneously or intravenously.

[00404] In certain embodiments, 0.13 mg/kg of ActRIIA-hFc (SEQ ID NO:7) is administered subcutaneously to a subject treated in accordance with the methods provided at an interval of once every 14 days. In certain embodiments, 0.26 mg/kg of ActRIIA-hFc (SEQ ID NO:7) is administered subcutaneously to a subject treated in accordance with the methods

provided at an interval of once every 14 days. In certain embodiments, 0.1 mg/kg of ActRIIA-hFc (SEQ ID NO:7) is administered intravenously to a subject treated in accordance with the methods provided at an interval of once every 14 days. In certain embodiments, 0.2 mg/kg of ActRIIA-hFc (SEQ ID NO:7) is administered intravenously to a subject treated in accordance with the methods provided at an interval of once every 14 days. In certain embodiments, 0.3 mg/kg of ActRIIA-hFc (SEQ ID NO:7) is administered subcutaneously to a subject treated in accordance with the methods provided at an interval of once every 28 days. In certain embodiments, 0.5 mg/kg of ActRIIA-hFc (SEQ ID NO:7) is administered subcutaneously to a subject treated in accordance with the methods provided at an interval of once every 28 days. In certain embodiments, 0.7 mg/kg of ActRIIA-hFc (SEQ ID NO:7) is administered subcutaneously to a subject treated in accordance with the methods provided at an interval of once every 28 days.

8.8 COMBINATION THERAPY

[00405] In certain embodiments, the methods provided herein are performed in combination with a second pharmaceutically active agent. Such combination therapy may be achieved by way of the simultaneous, sequential, or separate dosing of the individual components of the treatment. Additionally, when administered as a component of such combination therapy, the ActRII signaling inhibitor and the second pharmaceutically active agent may be synergistic, such that the daily dose of either or both of the components may be reduced as compared to the dose of either component that would normally be given as a monotherapy. Alternatively, when administered as a component of such combination therapy, the ActRII signaling inhibitor provided herein and the second pharmaceutically active agent may be additive, such that the daily dose of each of the components is similar or the same as the dose of either component that would normally be given as a monotherapy.

[00406] In certain embodiments, the ActRII signaling inhibitor provided herein is administered on the same day as a second pharmaceutically active agent. In certain embodiments, the ActRII signaling inhibitor is administered one, two, three, or more days before a second pharmaceutically active agent. In certain embodiments, the ActRII signaling inhibitor is administered one, two, three or more days after a second pharmaceutically active agent. In certain embodiments, the ActRII signaling inhibitor is administered within one, two, three or more weeks of a second pharmaceutically active agent.

[00407] In certain embodiments, the second pharmaceutically active agent is an antagonist of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin (*e.g.*, free

activin), Runx2, Alp, BSAP, CTX, and/or Osterix, such as an antibody or fragment thereof, a small molecule signaling inhibitor, an antisense nucleic acid, a small interfering nucleic acid, a dominant negative protein or fragment thereof. In certain embodiments, the second pharmaceutically active agent is an agonist of Klotho, alpha-SMA, MYOCD, Axin2 and/or Sm22-alpha, such as an antibody or fragment thereof or a small molecule.

[00408] In certain embodiments, the second pharmaceutically active agent is an active agent used to treat cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, and/or cardiovascular disease associated with and/or resulting from renal disease, such as, for example, an aldosterone signaling inhibitor, an angiotensin II receptor blocker, a beta-blocker, a calcium channel blocker, a cholesterol-lowering drug, digoxin, a diuretic, an inotropic therapy, potassium or magnesium, a vasodilator, and/or warfarin.

[00409] In certain embodiments, the second pharmaceutically active agent is an active agent used to treat chronic kidney disease, such as, for example, an angiotensin II receptor blocker, a beta-blocker, a calcium channel blocker, direct rennin signaling inhibitor, a diuretic, a vasodilator, erythropoietin therapy, iron replacement therapy, and/or vitamin D.

[00410] In certain embodiments, the methods provided herein are performed in combination with a method for treating or ameliorating cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, and/or cardiovascular disease associated with and/or resulting from renal disease, and/or chronic kidney disease.

8.9 PHARMACEUTICAL COMPOSITIONS

[00411] In certain embodiments, activin-ActRII antagonists (*e.g.*, ActRII polypeptides) are formulated with a pharmaceutically acceptable carrier for use with the methods described herein. For example, an ActRII polypeptide can be administered alone or as a component of a pharmaceutical formulation (therapeutic composition). The subject compounds may be formulated for administration in any convenient way for use in human or veterinary medicine. ActRII can be ActRIIA or ActRIIB.

[00412] In certain embodiments, the therapeutic methods provided herein include administering the composition (comprising an ActRII signaling inhibitor) systemically, or locally as an implant or device. When administered, the therapeutic composition for uses provided herein is in a pyrogen-free, physiologically acceptable form. Therapeutically useful agents other

than the ActRII antagonists which may also optionally be included in the composition as described above, may be administered simultaneously or sequentially with the subject compounds (*e.g.*, ActRII polypeptides, such as ActRIIA and / or ActRIIB polypeptides (*see*, Section 8.5)).

[00413] Typically, ActRII antagonists will be administered parenterally. Pharmaceutical compositions suitable for parenteral administration may comprise one or more ActRII polypeptides in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions for use in the methods described herein include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[00414] Further, the composition may be encapsulated or injected in a form for delivery to a target tissue site (*e.g.*, bone). In certain embodiments, compositions for use in the methods described herein may include a matrix capable of delivering one or more therapeutic compounds (*e.g.*, ActRIIA polypeptides) to a target tissue site (*e.g.*, bone), providing a structure for the developing tissue and optimally capable of being resorbed into the body. For example, the matrix may provide slow release of the ActRIIA polypeptides. Such matrices may be formed of materials presently in use for other implanted medical applications.

[00415] The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the subject compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are non-biodegradable and chemically defined, such as sintered

hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

[00416] In certain embodiments, the compositions for use in the methods described herein (comprising ActRII signaling inhibitor) can be administered orally, *e.g.*, in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of an agent as an active ingredient. An agent may also be administered as a bolus, electuary or paste.

[00417] In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), one or more therapeutic compounds described herein may be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[00418] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as

water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

[00419] Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol, and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[00420] The compositions described herein may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

[00421] It is understood that the dosage regimen will be determined by the attending physician considering various factors which modify the action of the compounds described herein (*e.g.*, ActRII polypeptides, such as ActRIIA and / or ActRIIB polypeptides (*see,* Section 8.5)). The various factors include, but are not limited to, amount of bone weight desired to be formed, the degree of bone density loss, the site of bone damage, the condition of the damaged bone, the subject's age, sex, and diet, the severity of any disease that may be contributing to bone loss, time of administration, and other clinical factors. Optionally, the dosage may vary with the type of matrix used in the reconstitution and the types of compounds in the composition. The addition of other known growth factors to the final composition, may also affect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, X-rays (including DEXA), histomorphometric determinations, and tetracycline labeling.

[00422] In certain embodiments, provided herein is gene therapy for the in vivo production of ActRII polypeptides. Such therapy would achieve its therapeutic effect by

introduction of the ActRII polynucleotide sequences into cells or tissues having the disorders as listed above. Delivery of ActRII polynucleotide sequences can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Preferred for therapeutic delivery of ActRII polynucleotide sequences is the use of targeted liposomes. The ActRII polypeptides can be ActRIIA and / or ActRIIB polypeptides (*see*, Section 8.5)).

[00423] Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. Retroviral vectors can be made target-specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody. Those of skill in the art will recognize that specific polynucleotide sequences can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing the ActRII polynucleotide. In a preferred embodiment, the vector is targeted to bone or cartilage.

[00424] Alternatively, tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

[00425] Another targeted delivery system for ActRII polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system for use in the methods described herein is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (*see e.g.*, Fraley, *et al.*, Trends Biochem. Sci., 6:77, 1981). Methods for efficient gene transfer using a liposome vehicle, are known in the art, *see e.g.*, Mannino, *et al.*, Biotechniques, 6:682, 1988. The composition of the liposome is usually a combination of phospholipids, usually in combination with steroids,

especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

[00426] Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine. The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art.

[00427] In certain embodiments, the ActRII signaling inhibitor is substantially pure in a pharmaceutical composition. Specifically, at most 20%, 10%, 5%, 2.5%, 1%, 0.1%, or at most 0.05% of the compounds in the pharmaceutical composition are compounds other than the ActRII signaling inhibitor and the pharmaceutical acceptable carrier.

8.10 KITS

[00428] Provided herein is a kit comprising one or more containers filled with one or more reagent to determine the level of one or more biomarkers described herein (*e.g.*, Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of one biomarker described herein (*e.g.*, Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of Runx2 in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of Alp in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of Snai1 in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of phosphosmad2 in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of Dkk1 in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of col1a1 in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of activin in a sample

obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of BSAP in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of CTX in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of osterix in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of Klotho in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of alpha-SMA in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of MYOCD in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of Sm22-alpha in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of phosphosmad3 in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of urinary protein in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of ActRIIA in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of Axin2 in a sample obtained from a subject described herein.

[00429] In certain embodiments, the kit comprises one or more reagent to determine the level of two biomarkers described herein (*e.g.*, two biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of three biomarkers described herein (*e.g.*, three biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of four biomarkers described herein (*e.g.*, four biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary

protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of five biomarkers described herein (*e.g.*, five biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of six biomarkers described herein (*e.g.*, six biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of seven biomarkers described herein (*e.g.*, seven biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of eight biomarkers described herein (*e.g.*, eight biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of nine biomarkers described herein (*e.g.*, nine biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of ten biomarkers described herein (*e.g.*, ten biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of eleven biomarkers described herein (*e.g.*, eleven biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a

subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of twelve biomarkers described herein (*e.g.*, twelve biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of thirteen biomarkers described herein (*e.g.*, thirteen biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of fourteen biomarkers described herein (*e.g.*, fourteen biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of fifteen biomarkers described herein (*e.g.*, fifteen biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of sixteen biomarkers described herein (*e.g.*, sixteen biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of seventeen biomarkers described herein (*e.g.*, seventeen biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments, the kit comprises one or more reagent to determine the level of eighteen biomarkers described herein (*e.g.*, eighteen biomarkers selected from the group consisting of Runx2, Alp, Snai1, phosphosmad2, Dkk1, col1a1, activin, BSAP, CTX, osterix, Klotho, alpha-SMA, MYOCD, Sm22-alpha, phosphosmad3, ActRIIA, Axin2, and urinary protein) in a sample obtained from a subject described herein. In certain embodiments,

the one or more reagent to determine the level of the biomarker is as described in Section 8.6.1. In certain embodiments, the kit further comprises ActRIIA-hFc (SEQ ID NO:7). In certain embodiments, the kit further comprises a second container comprising ActRIIA-hFc (SEQ ID NO:7).

9. EXAMPLES

[00430] The examples presented herein demonstrate that Runx2, Alp, BSAP, CTX, and Osterix mRNA levels are elevated and Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and Sm22-alpha mRNA levels can be decreased in certain forms of chronic kidney disease. These examples further demonstrate that treatment with an activin ligand trap decreases Runx2, Alp, CTX, ActRIIA, and/or Osterix mRNA levels and increases Klotho, alpha-SMA, MYOCD, Axin2, and/or Sm22-alpha mRNA levels, which is associated with a decrease in vascular calcification. Accordingly, the examples provided herein demonstrate that Runx2, Alp, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha can be used as a biomarker(s) for treating cardiovascular disease, vascular calcification, cardiovascular disease associated with and/or resulting from vascular calcification, cardiovascular disease associated with and/or resulting from renal disease, elevated levels of arterial stiffness, cardiovascular disease associated with and/or resulting from elevated levels of arterial stiffness, LVH, and/or cardiovascular disease associated with and/or resulting from LVH.

9.1 EXAMPLE 1. TREATMENT OF THE CKD-MBD WITH A LIGAND TRAP FOR THE ACTIVIN RECEPTOR TYPE 2A

[00431] CKD-MBD can comprise vascular calcification, an osteodystrophy, and stimulation of skeletal osteocyte FGF23 secretion at its inception, and hyperphosphatemia, develops later in the course to further stimulate vascular calcification. This example demonstrates that inhibition of ActRIIA signaling, a member of the TGFβ superfamily signaling through the activin type IIA receptor (ActRIIA), induced by CKD inhibits vascular calcification and prevents cardiac hypertrophy.

9.1.1 METHODS

[00432] CKD with hyperphosphatemia and 60% reduction in GFR (CKD-3) was induced at 14 weeks of age in a type 2 diabetes ldlr-/- mice, high fat fed model of vascular calcification. Some CKD mice were treated with an activin receptor type 2A (mActRIIA) ligand trap mActRIIA-Fc, injected IP weekly beginning at 22 weeks of age, and studied at 28 weeks. Aortic

Ca^{2+} levels, expression of osteoblastic and vascular smooth muscle proteins, skeletal histomorphometry and microCT imaging, serum chemistries and FGF23 and PTH levels were measured. Activin, Follistatin and Inhibin levels were measured by ELISA, RT-PCR and Western blots.

9.1.2 RESULTS

[00433] Circulating Activin levels were increased in two different kidney disease models: interstitial fibrosis and X-linked Alports (Fig. 1A and Fig. 1B). Moreover, Activin A mRNA levels were increased in the aorta and kidney of a CKD model (Fig. 2). Activin levels were increased in the vasculature and the circulation without changes in follistatin (Figure 2A, Figure 2B, and Figure 2C). CKD stimulated vascular calcification which was reduced below 22 week levels by treatment with an activin receptor type 2A (mActRIIA) ligand trap (mActRIIA-Fc; *see, e.g.*, U.S. Patent No. 8,173,601, the disclosure of which is hereby incorporated by reference in its entirety) (Fig. 3A, Fig. 3B, Fig. 3C, Fig. 3D, Fig. 5A, and Fig. 5B), and cardiac hypertrophy was prevented. CKD induced expression of aortic Runx2, osterix, and Alp message and protein, and these were reversed by treatment with an activin receptor type 2A (mActRIIA) ligand trap mActRIIA-Fc (Fig. 4A and Fig. 4B for Runx2 and Alp mRNA, respectively; Fig. 4F for Runx2 protein). CKD reduced Klotho and Sm22-alpha message and protein, and treatment with an activin receptor type 2A (mActRIIA) ligand trap mActRIIA-Fc normalized Klotho and Sm22-alpha expression (Fig. 4C and Fig. 4E for Klotho and Sm22-alpha mRNA, respectively; Fig. 4F for protein). Moreover, CKD reduced MYOCD message (Fig. 4D). In addition, CKD reduced alpha-smooth muscle actin (actin alpha-smooth muscle) protein (Fig. 4F). Further, CKD stimulated increased levels of calcium which were reduced by treatment with mActRIIA-Fc (Fig. 6A). Bone volume was increased by treatment with an activin receptor type 2A (mActRIIA) ligand trap mActRIIA-Fc, and osteoclast pit surface was decreased, but bone formation rates and osteoblast surfaces were not affected (Fig. 6B, Fig. 6C, and Fig. 6D). Hyperphosphatemia and FGF23 levels were not changed by treatment with an activin receptor type 2A (mActRIIA) ligand trap mActRIIA-Fc.

[00434] Treatment with an activin receptor type 2A (mActRIIA) ligand trap mActRIIA-Fc inhibited bone resorption and increased bone volume. Treatment with an activin receptor type 2A (mActRIIA) ligand trap mActRIIA-Fc inhibited Smad dependent signaling, blocked aortic osteoblastic transition, increased vascular smooth muscle protein levels and decreased CKD stimulated vascular calcification and cardiac hypertrophy.

9.2 EXAMPLE 2. CHRONIC KIDNEY DISEASE (CKD) STIMULATES AORTIC ENDOTHELIAL TO MESENCHYMAL TRANSITION (ENMT) WHICH CAUSES VASCULAR CALCIFICATION AND IS INHIBITED BY AN ACTIVIN LIGAND TRAP

[00435] The molecular mechanism underlying vascular calcification in chronic kidney disease is incompletely understood. However, activin levels are increased in CKD-stimulated vascular calcification. To assess the role of activins in CKD-stimulated vascular calcification, a recombinant fusion protein that binds a number of TGF-beta superfamily ligands was used in a mouse model of chronic kidney disease in a model of atherosclerosis and type-2 diabetes. The ActRIIA-Fc fusion protein consists of the extracellular domain of Activin Receptor IIA (ActRIIA) linked to an immunoglobulin 1 (IgG1) Fc domain and the protein acts as a ligand trap for TGF-beta family members like activin A, activin B, growth differentiation factor-11 (GDF11) and bone morphogenetic protein-10 (BMP-10).

[00436] It has previously been shown that kidney diseases causes vascular calcification by producing systemic Wnt inhibition during kidney repair. This example demonstrates that the vascular effects of CKD stimulated Wnt inhibition are stimulation of vascular activin as well as induction of Smad dependent EnMT, and that an activin receptor ligand trap, inhibits EnMT and vascular calcification.

9.2.1 METHODS

[00437] CKD with elevated Wnt inhibitors was induced in the mouse models for lineage tracing and vascular calcification. Activin, Follistatin and Inhibin levels were measured by ELISA, RT PCR and Western blots. Cell lineage tracing was performed in GNZ mice (Stoller et al, Genesis, 2008) bred to endothelial specific Tie2-Cre mice. Mice harboring knock in of GNZ express nuclear GFP and lacZ following Cre-mediated recombination.

9.2.2 RESULTS

[00438] Activin levels were increased in the vasculature and the circulation without changes in follistatin levels in mouse models of kidney disease and CKD stimulated vascular calcification. CKD induced expression of GFP and lacZ in cells of the adventia and media of GNZ; Tie2-Cre CKD mice compared to GNZ; Tie2-Cre mice with normal kidney function wherein the GFP and lacZ were limited to the aortic endothelium. Tie2 is an endothelial lineage specific receptor, and this demonstrates that CKD induces aortic EnMT (Fig. 7A, Fig. 7B, Fig.

7C, and Fig. 7D). CKD stimulated aortic EnMT produced decreased vascular smooth muscle function, osteoblastic transition and calcification. Treatment with an activin receptor type 2A (mActRIIA) ligand trap (mActRIIA-Fc; *see, e.g.*, U.S. Patent No. 8,173,601, the disclosure of which is hereby incorporated by reference in its entirety), inhibited Smad dependent signaling, blocked aortic osteoblastic transition, increased vascular smooth muscle function and decreased CKD stimulated vascular calcification. Thus, CKD induced vascular activin and EnMT, and treatment with an activin receptor type 2A (mActRIIA) ligand trap decreased activin signaling inhibiting vascular dedifferentiation, osteoblastic transition and vascular calcification.

9.3 EXAMPLE 3. INITIAL SIGNAL-SEEKING QUANTITATIVE COMPUTED TOMOGRAPHY RESULTS FOR BONE MASS AND VASCULAR CALCIFICATION IN HEMODIALYSIS SUBJECTS TREATED WITH ESCALATING DOSES

[00439] High turnover renal osteodystrophy (ROD) is marked by increased cortical porosity, higher trabecular bone mass, and increased risk of fracture. ActRIIA-Fc, an activin A RIIA-IgG1 fusion protein ligand trap under study for the correction of anemia in hemodialysis (HD) subjects, blocks activin A signaling and may reduce osteoclastogenesis and promote osteoblast maturation in bone. The current analysis in HD subjects evaluated the effect of ActRIIA-Fc on bone mineral density (BMD) and vascular calcification using quantitative computed-tomography (QCT).

9.3.1 METHODS

[00440] Hemodialysis subjects treated with an ActRIIA signaling inhibitor who were erythropoietin-stimulating agent (ESA)-responsive were washed out of ESA effect until hemoglobin (Hb) was <10 g/dL, then randomized to treatment with an ActRIIA signaling inhibitor at the following doses: 0.3 mg/kg (n=9), 0.5 mg/kg (n=8), 0.7 mg/kg (n=6), or placebo (PBO; n=7) subcutaneously every 28 days for up to 8 dose cycles. Subjects were assessed for effects on Hb, bone mineral density (BMD), and biomarkers of bone turnover. Treatment failures (Hb <9 g/dL) were rescued with ESA and/or red blood cell transfusion. Quantitative computed tomography (QCT) of the hip and lumbar spine, was obtained at baseline and after the 225-day treatment phase. Biomarkers, BSAP and CTX, are measured at baseline and after dose cycles 3, 5, and 7.

[00441] QCT of the hip, lumbar spine, and abdominal aorta were obtained at baseline and after the 225-day treatment phase. Subjects were positioned supine on a Mindways calibration

phantom (Model 3; Mindways Software, Inc., Austin, TX). The slice thickness of 2.5 mm and 512 x 512 matrix is reconstructed using a standard soft-tissue kernel. Mindways analysis software (version 5.0.3) was used to assess volumetric BMD (vBMD). Trabecular vBMD (mg/cm3) was determined for 2 vertebrae within L1-4 (typically L1-2). The left proximal femurs were analyzed for vBMD of the cortical, trabecular, and integral bone compartments of the total hip, femoral neck, and trochanteric regions.

[00442] Vascular calcification of the abdominal aorta was assessed using software that semi-automatically segments the area and volume of calcifications within the region adjacent to the top of L1 through the bottom of L4. The number and location of slices was maintained across visits per subject. Agatston and square root transformed volumetric scores were determined as described in Agatston *et al.*, *J Am Coll Cardiol.* 1990; 15:827-832 and Hokanson *et al.*, *AJR AM J Roentgenol.* 2004;182:1327-1332. Lower total Agatston and square root transformed total volume scores (mm3) indicate lower levels of vascular calcification (VC).

[00443] All image quality control and blinded analyses are performed centrally by PAREXEL imaging (PAREXEL International Corp. Waltham, MA).

[00444] Biomarkers, including bone-specific alkaline phosphatase (BSAP), pro-collagen type 1 N-terminal propeptide (P1NP), and C-terminal type 1 collagen telopeptide (CTX), were measured at baseline and after dose cycles 1, 3, 5, and 7.

9.3.2 RESULTS

[00445] A total of 31 subjects were randomized and receive more than one dose of study medication.

[00446] **Table 2**. Randomized Subjects and QCT Analysis Subset

		ActRIIA-Fc		
Subjects, n	**Placebo**	**0.3 mg/kg**	**0.5 mg/kg**	**0.7 mg/kg**
Randomized and received ≥1 dose of study medication	8	9	8	6
QCT measures at baseline and Day 225	3	6	5	2

[00447] The subject disposition is described in Fig. 8, including the subjects with paired QCT measurements at baseline and Day 225.

[00448] Most subjects were discontinued from study treatment after treatment failure requiring rescue (generally because of Hb <9 g /dL); no subject discontinues treatment because of an adverse event (AE).

[00449] Of the 16 subjects with paired QCT measurements, 9 required rescue therapy during the treatment phase, 8 of whom required rescue within the first 3 dose cycles.

[00450] Among subjects with paired QCT measurements, baseline demographic and clinical characteristics were generally similar across treatment groups (Table 3); however, there was a substantially longer time on dialysis in the placebo group, which was also the youngest group. There were also differences between groups in baseline biomarker and Agatston scores (Table 4).

[00451] **Table 3.** Baseline Demographic and Clinical Characteristics of Subjects With Paired QCT Measurements

		ActRIIA-Fc		
	Placebo n=3	**0.3 mg/kg n=6**	**0.5 mg/kg n=5**	**0.7 mg/kg n=2**
Age, mean, years	54.0	57.3	60.8	69.0
Female, n (%)	0 (0.0)	4 (66.7)	0 (0.0)	1 (50.0)
Race, n (%)				
White	1 (33.0)	3 (50.0)	3 (60.0)	1 (50.0)
Black	1 (33.0)	3 (50.0)	2 (40.0)	1 (50.0)
Asian	1 (33.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ethnicity, n (%)				
Hispanic	0 (0.0)	2 (33.0)	3 (60.0)	1 (50.0)
Non-Hispanic	3 (100.0)	4 (66.7)	2 (40.0)	1 (50.0)
Postdialysis weight, mean, kg	70.4	80.3	79.4	84.4
Body mass index, mean, kg/m^2	25.4	27.7	26.9	29.2
Diabetes, n (%)	2 (66.7)	5 (83.3)	5 (100.0)	2 (100.0)
Parathyroidectomy, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Time on dialysis, mean, months	101.2	43.3	22.4	11.3
Non-calcium phosphate binder, n (%)*	3 (100.0)	3 (50.0)	3 (60.0)	0 (0.0)
Calcium-based phosphate binder, n (%)*	1 (33.3)	4 (66.7)	4 (80.0)	2 (100.0)
Calcimimetic, n (%)*	1 (33.3)	2 (33.3)	0 (0.0)	0 (0.0)
1,25-OH vitamin D analog, n (%)	2 (66.7)	5 (83.3)	2 (40.0)	2 (100.0)

[00452] **Table 4.** Mean Baseline Biomarker, Areal BMD, and Agatston Scores of Subjects With Paired QCT Measurements

		ActRIIA-Fc		
	Placebo n=3	**0.3 mg/kg n=6**	**0.5 mg/kg n=5**	**0.7 mg/kg n=2**
Baseline whole PTH, pg/mL	261.4	104.8	135.5	100.4
BSAP, µg/L	28.3	18.0	13.2	7.5
P1NP, ng/mL	623.7	376.2	468.4	308.0
CTX, pg/mL	3,417.7	2,062.5	2,266.8	1,377.0
Total hip integral BMD, mg/cm^3	279.4	306.2	268.9	285.4
Femoral neck cortical BMD, mg/cm^3	653.1	666.9	593.1	566.5
Mean spine (L1, L2) BMD, mg/cm^3	123.1	125.6	149.1	150.6
VC total Agatston score*	8,665.0	9,472.7	3,618.5	823.2

PTH=paraythroid hormone.
*Lower Agatston scores indicate lower levels of vascular calcification.

[00453] Table 5 provides the percent change from baseline in hip integral, femoral neck cortical, hip cortical, and lumbar spine areal BMD measurements.

[00454] **Table 5.** Baseline and Percent Change From Baseline in Hip Integral, Femoral Neck Cortical, and Lumbar Spine Areal BMD

		ActRIIA-Fc		
	Placebo n=3	**0.3 mg/kg n=6**	**0.5 mg/kg n=5**	**0.7 mg/kg n=2**
Total hip integral				
Baseline BMD, mg/cm^3	279.4	306.2	268.9	285.4
% Change from baseline BMD (n)	-0.2 (3)	2.5 (5)	4.0 (5)	-1.8 (1)
Femoral neck cortical				
Baseline BMD (mg/cm^3)	653.1	666.9	593.1	565.5
% Change from baseline BMD (n)	-0.9 (3)	-1.4 (5)	1.6 (5)	3.0 (1)
Total hip, cortical				
Baseline BMD (mg/cm^3)	708.6	668.4	647.3	635.6
% Change from baseline BMD (n)	-0.1 (3)	-1.1 (5)	0. 5 (5)	2.7 (1)
Mean lumbar spine (L1, L2)				
Baseline BMD (mg/cm^3)	123.1	125.6	149.1	150.6
% Change from baseline BMD (n)	12.6 (3)	8.0 (6)	0.5 (5)	-2.7 (2)

[00455] A 2% increase in cortical bone may reduce fracture risk. ACTRIIA signaling inhibitor treatment was associated with a dose-dependent increase in the proportion of subjects with a greater than or equal to 2% increase in femoral neck cortical bone (Table 5 and Fig. 9A.

[00456] In high-turnover ROD, trabecular bone mass increases (i.e., poorer quality bone), was measured by lumbar spine BMD, without a reduction in vertebral fracture rates in ESKD compared with the general population. Treatment with ACTRIIA prevented the increase in trabecular bone mass in the lumbar spine (Table 5 and Fig. 9B).

[00457] The changes from baseline in abdominal aorta total Agatston scores are provided in Table 6. Categories of change in the total Agatston score and square root transformed total volume score are shown in Fig. 9 and Table 7.

[00458] **Table 6.** Baseline and Change From Baseline in Abdominal Aorta Total Agatston Score and Square Root Transformed Total Volume Score

		ActRIIA-Fc		
	Placebo n=3	**0.3 mg/kg n=6**	**0.5 mg/kg n=5**	**0.7 mg/kg n=2**
Abdominal aorta total Agatston score				
Baseline total Agatston score	8,665	9,473	3,619	823
Change from baseline total Agatston score	1,050.4	8,578.9	225.7	43.4
% Change from baseline total Agatston score	58.4	24.9	17.3	3.4
Square root transformed total volume score				
Baseline square root of total volume, mm^3	52.0	46.2	33.1	16.9
Change from baseline square root of total volume, mm^3	4.5	11.1	1.2	0.4

*Lower total Agatston and square root transformed total volume scores indicate lower levels of vascular calcification.

[00459] Eleven subjects had baseline Agatston scores that were distributed between 10 and 10,000. Five subjects may be considered outliers at baseline (low outliers: 0.0, 1.1, and 1.4 Agatston scores; high outliers: 21,033 and 44,356 Agatston scores). Therefore, the Agatston scores were analyzed without the outliers (Table 7 and Fig. 10).

[00460] **Table 7.** Baseline and Change in Total Agatston Score Excluding 5 Outliers

		ActRIIA-Fc		
	Placebo n=1	**0.3 mg/kg n=4**	**0.5 mg/kg n=4**	**0.7 mg/kg n=2**
Re-baseline total Agatston score, excluding 5 outliers	4,960	3,120	4,523	823
Change from baseline total Agatston score, excluding 5 outliers	2,711.3	287.7	282.0	43.4
% Change from baseline, excluding 5 outliers	54.7	9.0	5.0	3.4

*Lower total Agatston and square root transformed total volume scores indicate lower levels of vascular calcification.

[00461] Subjects were analyzed by duration of exposure to the ActRIIA signaling inhibitor. Subjects were analyzed by exposure status: ActRIIA-Fc subjects receiving up to 3 doses, regardless of dose level (n=6), and ActRIIA-Fc subjects receiving >3 doses, regardless of dose level (n=7). The placebo group (n=3) was unchanged. Other than gender and Agatston score, key baseline characteristics were generally similar between those receiving ≤3 doses of ActRIIA-Fc and those receiving >3 doses (Table 8). An exposure effect on VC remained intact in all ActRIIA-Fc subjects (Table 9), as well as when excluding outliers (Table 10).

[00462] **Table 8.** Baseline Demographic and Clinical Characteristics of Subjects, by ActRIIA-Fc Exposure

	ActRIIA-Fc	
	≤3 Doses n=6	**>3 Doses n=7**
Age, mean, years	59.0	61.7
Female, n (%)	4 (66.7)	1 (14.3)
Race, n (%)		
White	3 (50.0)	4 (57.1)
Black	3 (50.0)	3 (42.9)
Asian	0 (0.0)	0 (0.0)
Body mass index, mean, kg/m^2	25.7	29.3
Diabetes, n (%)	5 (83.3)	7 (100.0)
Time on dialysis, months	31.3	24.0
Calcium-based phosphate binder, n (%)	4 (66.7)	6 (85.7)
Calcimimetic, n (%)	1 (16.7)	1 (14.3)
1,25-OH vitamin D analog, n (%)	4 (66.7)	5 (71.4)
Baseline whole PTH, mean, pg/mL	104.5	125.7
BSAP, mean, µg/L	19.8	10.0
P1NP, mean, ng/mL	432.2	374.6
CTX, mean, pg/mL	2,410.3	1,714.4
VC total Agatston score, mean	8,350	3,782
Square root of volume, mm^3	38.2	35.3

[00463] **Table 9.** Baseline and Change From Baseline in Total Agatston Score and Square Root Transformed Total Volume Score, by ActRIIA-Fc Exposure*

	ActRIIA-Fc	
	≤3 Doses n=6	>3 Doses n=7
Baseline total Agatston score	8,350	3,782
Change from baseline total Agatston score	8,461.4	274.3
% Change from baseline total Agatston score	33.7	5.8
Baseline square root of total volume, mm^3	38.2	35.3
Change from baseline square root of total volume, mm^3	10.6	1.4

*Lower total Agatston scores and square root transformed total volume scores indicate lower levels of vascular calcification.

[00464] **Table 10.** Baseline and Change From Baseline in Total Agatston Score, Excluding 5 Outliers, by ActRIIA-Fc Exposure*

	ActRIIA-Fc	
	≤3 Doses n=3	>3 Doses n=7
Re-baseline total Agatston score, excluding 5 outliers	1,914	3,782
Change from baseline total Agatston score, excluding 5 outliers	148.4	274.3
% Change from baseline, excluding 5 outliers	7.5	5.8

*Lower total Agatston scores and square root transformed total volume scores indicate lower levels of vascular calcification.

[00465] Bone turnover biomarkers were evaluated. The placebo group had the highest PTH, BSAP, P1NP, and CTX levels at baseline (Table 4). No consistent changes from baseline in phosphorous, PTH, FGF-23, or sclerostin levels were observed, except that PTH was lower at all time points than at baseline in the placebo group (-15.2 to -100.7 ng/L). For bone biomarkers, no clear changes were seen in percent change in the formation markers BSAP and P1NP; percent change in the resorption marker CTX is shown in Fig. 11.

More subjects receiving 0.5 mg/kg of the ACTRIIA signaling inhibitor had meaningful, persistent decreases in the resorption marker CTX.

9.3.3 CONCLUSIONS

[00466] Of the 30 randomized subjects, 13 had paired QCT assessments (n=3, 6, and 4 for PBO, 0.3 mg/kg, and 0.5 mg/kg, respectively). Relative BMD changes from baseline for PBO, 0.3 mg/kg, and 0.5 mg/kg were -0.9%, -1.4%, and +1.9% of the femoral neck cortical, and +12.6%, +8.0%, and -1.9% of the lumbar spine, respectively. Biomarker changes with 0.5 mg/kg suggest a beneficial antiresorptive effect.

[00467] These data demonstrate an emerging dose effect with ActRIIA-Fc 0.5 mg/kg, which appears to reverse the effects of high turnover ROD on cortical and cancellous bone. PBO results are as expected.

9.4 EXAMPLE 4. KIDNEY DISEASE PRODUCED CIRCULATING RENAL REPAIR FACTORS CAUSE CARDIOVASCULAR DISEASE

[00468] Kidney diseases are associated with an extremely high mortality, which is related to their production of cardiovascular disease (Sarnak, M.J. *et al.*, 2003. Circulation 108:2154-2169.). An ancillary study of the ongoing REGARDS project, demonstrated that in patients having sustained a coronary artery event (cardiac ischemia or non-fatal myocardial infarction) the incidence of second cardiovascular events over a median follow-up of 4 years was 35% in patients with kidney disease, compared to 19% in a high risk group (smokers, diabetes, hypercholesterolemia), and that the incidence of all-cause mortality in the high risk group was half that of the chronic kidney disease (CKD) group (Baber, U., *et al.*, 2013. Am Heart J 166:373-380.). The causes of the increased cardiovascular risk associated with kidney diseases reside in a syndrome named in 2006 –the chronic kidney disease – mineral bone disorder (CKD-MBD) (Moe, S., *et al.*, 2006. Kidney Int 69:1945-1953.). In the CKD-MBD, three novel cardiovascular risk factors have been discovered (Block, G.A., *et al.*, 1998. Am J Kidney Dis 31:607-617; Blacher, J., *et al.*, 2001. Hypertension 38:938-942; Gutierrez, O.M., *et al.*, 2008. New Engl J Med 359:584-592.), and their risk factor status confirmed in the general population (Dhingra, R., *et al.*, 2007. Arch Intern Med 167:879-885; Matsushita, K., *et al.*, 2014. Journal of the American Society of Nephrology; Dalal, M., *et al.*, 2011. European Journal of Endocrinology 165:797-803.). These are hyperphosphatemia, vascular calcification, and elevated fibroblast growth factor 23 (FGF23) levels. The CKD-MBD begins in the early stages of CKD (stage 2) (Fang, Y., *et al.*, 2014. Kidney Int 85:142-150; Pereira, R.C., *et al.*, 2009. Bone 45:1161-1168; Fang, Y., *et al.*, 2009. J Am Soc Nephrol 20:36A; Hu, M.C., *et al.*, 2011. J Am Soc Nephrol 22:124-136.) consisting of vascular dedifferentiation/calcification, an osteodystrophy, loss of

Klotho and increased FGF23 secretion (Fang, Y., *et al.*, 2014. Kidney Int 85:142-150.), but while progress into the causes of the CKD-MBD in early kidney failure have been made (Hu, M.C., *et al.*, 2011. J Am Soc Nephrol 22:124-136; Fang, Y., *et al.*, 2014. J Am Soc Nephrol 25:1760-1763; de Oliveira, R.B., *et al.*, 2013. Nephrology Dialysis Transplantation 28:2510-2517; Sabbagh, Y. 2012. J. Bone Miner. Res. 27:1757-1772.), they are mostly unknown.

[00469] It has been demonstrated that kidney diseases reactivate developmental programs involved in nephrogenesis during disease stimulated renal repair (Surendran, K., *et al.*, 2002. Am J Physiol Renal Physiol 282:F431-F441; Surendran, K., *et al.*, 2005. J Am Soc Nephrol 16:2373-2384; Maeshima, A., *et al.*, 2001. Cytokine & Growth Factor Reviews 12:289-298; Terada, Y., *et al.*, 2003. Journal of the American Society of Nephrology 14:1223-1233; Kawakami, T., *et al.*, 2013. J Pathol 229:221-231.). Among the nephrogenic factors reactivated in renal repair, the Wnt (portmanteau of Wingless and Integrated) family is critical for tubular epithelial reconstitution (Terada, Y., *et al.*, 2003. Journal of the American Society of Nephrology 14:1223-1233; Kawakami, T., *et al.*, 2013. J Pathol 229:221-231; Rinkevich, Y., *et al.*, 2014. Cell Reports 7:1270-1283.). In the control of Wnt function, canonical signaling transcriptionally induces the expression of a family of Wnt inhibitory proteins which are secreted proteins that serve to restrict the distances of Wnt stimulation to autocrine or paracrine factors (Niida, A., *et al.*, 2004. Oncogene 23:8520-8526; Niehrs, C. 2006. Oncogene 25:7469-7481; Reya, T., *et al.*, 2003. Nature 423:409-414; Chamorro, M.N., *et al.*, 2004. The EMBO Journal 24:73-84; Gonzalez-Sancho, J.M., 2004. Oncogene 24:1098-1103.). The Wnt inhibitors are circulating factors, and the Wnt inhibitor family includes the Dickkopfs (Dkk), the secreted frizzled related proteins (Sfrps), sclerostin, sostDoc 1, crescent, Wnt inhibitor factor 1 (Wif1) and Icat (Niehrs, C. 2006. Oncogene 25:7469-7481). Various forms of kidney disease increase renal expression of Wnt inhibitors and increase their levels in the circulation (Fang, Y., *et al.*, 2014. J Am Soc Nephrol 25:1760-1763; Surendran, K., *et al.*, 2005. J Am Soc Nephrol 16:2373-2384.).

[00470] Neutralization of a key Wnt inhibitor elevated in the circulation in CKD, Dkk1, inhibits CKD induced vascular dedifferentiation, vascular calcification, and renal osteodystrophy (Fang, Y., *et al.*, 2014. J Am Soc Nephrol 25:1760-1763.). This effect is surprising since Wnt signaling in the vascular smooth muscle is implicated in stimulating osteoblastic transition and vascular calcification (Al-Aly, Z., *et al.*, 2007. Arteriosclerosis, Thrombosis, and Vascular Biology 27:2589-2596; Shao, J.S., *et al.*, 2005. Journal of Clinical Investigation 115:1210-1220.). However, recent studies demonstrate that Dkk1 mediated inhibition of aortic Wnt7b

stimulates smad-mediated aortic endothelial-mesenchymal transition (EnMT) and vascular calcification (Cheng, S.-L., *et al.*, 2013. Arteriosclerosis, Thrombosis, and Vascular Biology 33:1679-1689.). EnMT is a developmental physiologic process involved in the development of the cardiac valves, the cardiac septum and the aortic root (Eisenberg, L.M., and Markwald, R.R. 1995. Circulation Research 77:1-6; Camenisch, T.D., *et al.*, 2002. Developmental Biology 248:170-181.), and it may (Zeisberg, E.M., *et al.*, 2007. Nat Med 13:952-961.) or may not (Moore-Morris, T., *et al.*, 2014. The Journal of Clinical Investigation 124:2921-2934.) contribute to cardiac fibrosis in various adult disease states. This factor investigates whether other factors involved in attempted renal repair during kidney disease derive from the TGFβ superfamily and are increased in the circulation during CKD.

9.4.1 METHODS

9.4.1.1 Production of animal models

[00471] The atherosclerotic *low density lipoprotein receptor*-deficient *(ldlr-/-)* males (C57Bl/6J background) were purchased from Jackson Laboratories and fed high fat diet (42% calories from fat) (Teklad #) beginning at 12 weeks of age. The mice are obese, insulin resistant at 22 weeks of age, diabetic at 28 weeks of age and hypercholesterolemic.

[00472] A two-step procedure was utilized to create chronic kidney disease as described previously (Davies, M.R., *et al.*, 2003. J Am Soc Nephrol 14:1559-1567; Davies, M.R., *et al.*, 2005. J Am Soc Nephrol 16:917-928.). Electrocautery was applied to the right kidney through a 2 cm flank incision at 12 weeks post natal, followed by left total nephrectomy at 14 weeks of age. The intensity of the cautery was varied to produce moderate (CKD-3) renal injury that was confirmed by inulin clearances at age 20 weeks (Fig. 12A). Control animals received sham operations in which the appropriate kidney was exposed and mobilized but not treated in any other way. Five groups of mice were used in this study (Fig. 15B). The first group was wild type C57Bl/6J mice fed a regular chow diet (WT). This was the normal renal function and diet group used for normative control values. The second group was *ldlr-/-* mice that were fed a high fat diet and sham operated (Sham). This group had normal renal function, and it served as the control group to determine the effect of kidney disease. The third group was *ldlr-/-* mice with GFR reduced equivalent to human CKD stage 3 fed high fat diet (CKD-3) with euthanasia at 22 weeks, the baseline vascular calcification group (CKD-3). The fourth group was *ldlr-/-* mice with CKD-3 receiving subcutaneous injections of vehicle twice a week beginning at 22

weeks until euthanasia at 28 weeks (CKD-3 V). The fifth group was *ldlr-/-* mice with CKD-3 receiving subcutaneous injections of mActRIIA-Fc (Celgene, Summit, NJ), 10 mg/kg twice a week beginning at 22 weeks until euthanasia at 28 weeks (CKD-3 mActRIIA-Fc).

[00473] The second model of CKD used was the murine homolog of X-linked Alport's syndrome, which is a deficiency in the gene for the α5 chain of type IV collagen, COL4A5 (Rheault, M.N., *et al.*, 2004. Journal of the American Society of Nephrology 15:1466-1474.). This is a model of spontaneous kidney disease, and it was used throughout the results to confirm the effects of renal ablation induced CKD. Breeding pairs were purchased from Jackson Laboratories and were bred for experiments. Hemizygote males spontaneously developed kidney disease comparable with human CKD stage 3-4 at 200 days after birth.

[00474] The third model of CKD used was renal ablation, similar to the *ldlr-/-* protocol, in a transgenic mouse line used for cellular lineage tracing, the GNZ mouse (Fig.14). GNZ reporter female mice (Stoller, J.Z., *et al.*, 2008. Genesis (New York, N.Y. 2000) 46:200-204.) and Tek-Cre transgenic male mouse (Koni, P.A., *et al.*, 2001. The Journal of Experimental Medicine 193:741-754.) were purchased from Jackson Laboratories and were bred to produce GNZ / Tek-Cre + mice for experiments. GNZ/ Tek-Cre – littermates served as negative controls. Mouse genotyping was performed by using specific primers recommended for GNZ and Tek-Cre mouse strains by manufacturer. In all cases, euthanasia was performed under anesthesia. Intraperitoneal anesthesia (xylazine 13 mg/kg and ketamine 87 mg/kg) was used for all procedures. Saphenous vein blood samples were taken 1 week following the second surgery to assess baseline post-surgical renal function. At the time of sacrifice, blood was taken by intracardiac stab, and the heart and aorta dissected en bloc.

9.4.1.2 Inulin clearances

[00475] Inulin clearances at 20 weeks or 26 weeks, if euthanasia was at 28 weeks, were performed according to manufacturer instructions (BioPal Inc., Worcester, MA).

9.4.1.3 Chemical Calcification Quantitation

[00476] Aorta and hearts were dissected at sacrifice, and all extraneous tissue removed by blunt dissection under a dissecting microscope. Tissues were desiccated for 20-24 hours at 60° C, weighed and crushed to a powder with a pestle and mortar. Calcium was eluted in 1N HCL for 24 hours at 4°C. Calcium content of eluate was assayed using a cresolphthalein complexone method (Sigma, St Louis), according to manufacturer's instructions, and results were corrected for dry tissue weight.

9.4.1.4 Blood tests

[00477] Serum was analyzed on the day of blood draw for blood urea nitrogen (BUN), calcium, and phosphate by standard autoanalyzer laboratory methods. Plasma activin (Fitzgerald Industries, Acton, MA), follistatin (MYBIOSOURCE Inc., San Diego, CA) and follistatin-like 3 (MYBIOSOURCE Inc.) levels were determined in ELISA assays. Serum Dkk1 levels were analyzed with an ELISA (R&D Systems, Minn., MN). For the ELISA assays, blood was drawn by saphenous vein or cardiac puncture at the time of euthanasia. All blood samples were placed on ice at collection. Platelet poor EDTA plasma samples were made by a 2-step centrifugation at 6000 rpm for 5 minutes and 14000 rpm for 2 minutes both at 4°C. Samples were stored frozen at – 20° C or below until being used.

9.4.1.5 Histology and immunohistochemistry

[00478] Aortic, kidney and cardiac tissues were fixed in 10% neutral buffered formalin overnight, then transferred to 70% ethanol at 4 °C, embedded in paraffin and 5 micron sections were prepared. Slides were deparaffinized in xylene and dehydrated in a graded ethanol series and then rehydrated. Mason Trichome staining was used to detect kidney and heart fibrosis and Alizarin red staining was used to detect calcification, according to a standard protocol (Gregory, C.A., *et al.*, 2004. *Analytical Biochemistry* 329:77-84.). For immunohistochemical staining, endogenous peroxidase was blocked with 3% H O in methanol for 15 min. Non-specific binding was blocked with avidin and biotin blocking agents (Vector laboratories, Burlingame, CA, USA) for 30 min, then with 3% normal donkey serum for 20 min. After washing with PBS, the slides were incubated with primary antibody at 4 °C overnight, and followed by incubation with biotinylated secondary antibody (Vector laboratories) at room temperature for one hour, then the strepatividin – conjugated perioxidase staining was performed using DAB kit (SK-4100, Vector laboratories). For double immunofluorescent staining, sections were blocked in 20% goat serum and sequentially stained with first primary and secondary antibodies and second primary and secondary antibodies using goat-anti rabbit Alexa 488 and Alexa 568 secondary antibodies 1:400 (Life Technologies, A11008 and A11011) or TSA kit (Life Technologies, T 20932) for signal amplification according manufacture instructions. When primary antibodies from same species were used for double staining, slides were heated 5 min in citrate buffer in microwave before second staining (Toth and Mezey, 2007). Primary antibodies were used in this study for immunostaining: rabbit polyclonal anti-ActRIIA antibody 1:250 (Abcam, ab 135634), rabbit polyclonal anti-Inhibin beta A antibody 1:100 (Santa Cruz, sc-50288), rabbit polyclonal anti-

CD31 antibody 1:50 (Abcam, ab28364) and rabbit polyclonal anti-GFP antibodies 1:1000 (Abcam, ab6556).

9.4.1.6 RT-PCR

[00479] RNA was extracted from aortas and cell cultures using RNeasy Mini Kits (Qiagen, Valencia, CA). 1 μg of total RNA was DNase treated reverse transcribed using iScript cDNA synthesis kit from Bio-Rad (Hercules, Ca) according to manufacturer's instructions. Primers were designed using Vector NTI (Invitrogen, Grand Island, NY) or Primer Express software. A Perkin-Elmer DNA Thermal Cycler was used to perform the reaction. Following reverse transcription performed as above, real time was performed using the StepOne Plus real time PCR instrument (AB), SYBR Green from Sigma (St. Louis) and the PCR kit from Invitrogen. Each reaction was performed in triplicate at 95°C, 45 sec, and 60°C, 30 sec, and 60 sec at 72°C for 40 cycles. This was followed by a melt cycle, which consisted of stepwise increase in temperature from 60°C to 95°C. A single predominant peak was observed in the dissociation curve of each gene, supporting the specificity of the PCR product. Ct numbers (threshold values) were set within the exponential phase of PCR and were used to calculate the expression levels of the genes of interest. B2m was used as an internal standard and used to normalize the values. A standard curve consisting of the Ctversus log cDNA dilutions (corresponding to the log copy numbers) was generated by amplifying serial dilutions of cDNA corresponding to an unknown amount of amplicon. Negative controls were performed by inactivating the reverse transcriptase by boiling for 5 min prior to RT-PCR to insure that genomic DNA was not amplified.

9.4.1.7 Immunoblotting (Western analyses)

[00480] Whole-cell lysate protein were prepared from kidney and aorta of the mice by RIPA Lysis Buffer (Thermo Scientific) containing a protease inhibitor cocktail (Santa Cruz). Lysates (20 μg) were loaded in 8-12% SDS-PAGE gels and immunoblotted with antibodies to inhibin β-A (Santa Cruz), α-tubulin (Santa Cruz), snai 1 (Cell Signaling), alpha-SMA (Sigma), Runx2 (Cell Signaling), MYOCD (Santa Cruz), ACVRL1 (Origene), ACVR1 (Cell Signaling), Erk1/2 (Cell Signaling), phospho-Erk1/2 (Cell Signaling), ACVR1B (Origene), Col1a1 (Santa Cruz), Smad2/3 (Cell Signaling), or phospho-Smad2/3 (Cell Signaling). In immunoprecipitation (IP) assay, the same whole-cell lysate protein was used. To reduce nonspecific binding, the samples were pre-cleared using pre-washed protein A agarose beads (Cell Signaling). Pre-cleared samples were incubated overnight with the phospho-serine antibody (Abcam). IP-

antibody complexes were then captured on protein A agarose beads, and proteins were detected by immunoblotting analysis.

9.4.1.8 Human Studies

[00481] Subjects were enrolled after giving informed consent in accordance with guidelines from the Declaration of Helsinki. Subjects were eligible if they were greater than 18 years of age and had stage 3 CKD (estimated GFR 30-59 ml/min/1.73m^2 using the Modification of Diet in Renal Disease study equation (Levey, A.S., *et al.*, 1999. Ann Intern Med 130:461-470.). Exclusion criteria included pregnancy, bone disease, myocardial infarction, congestive heart failure, diastolic dysfunction or severe hypertension. Members of the research team identified their clinic patients that satisfied the inclusion criteria and were interested in the study. Medical records were reviewed to determine final eligibility before enrollment. Blood samples were obtained from each subject at the baseline visit and after 12 months of treatment. There was intersubject variation in the time of day that blood samples were obtained. Aliquots of plasma were generated from each sample and used immediately for biochemical testing or frozen at 80°C. Plasma levels of activin were measured in duplicate using commercially available ELISA kits, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

9.4.1.9 Statistics

[00482] Statistical analysis was performed using ANOVA. All data are expressed as mean±SD, unless other specified in the figure legend. Differences between groups were assessed post hoc using Fisher LSD method and considered significant at $p < 0.05$. Analyses were performed using Sigma Stat statistical software (Point Richmond, CA). Data for all groups represent an "n" of 7-15. For real time PCR analysis minimum 3 samples were used in each experimental group. Unpaired t-tests were used to compare CKD-3 mice treated with mActRIIA-Fc versus vehicle-treated CKD-3 V mice or sham-operated mice (Sham). In Fig. 6, the boxes represent median and interquartile ranges (from 25th to 75th percentile), and error bars present 1.5- fold of the interquartile range below 25th and above 75th percentile. Means were compared using ANOVA Holm –Sidak method for multiple comparison $p<0.05$ as critical level for significant difference.

9.4.2 RESULTS

9.4.2.1 Kidney function in models of CKD

[00483] CKD analogous to human stage 3 CKD (CKD-3) was induced in three experimental models. The first model was a model of CKD-stimulated atherosclerotic vascular calcification: the *ldlr-/-* high fat-fed mouse (Fang, Y., *et al.*, 2014. Kidney Int 85:142-150; Fang, Y., *et al.*, 2014. J Am Soc Nephrol 25:1760-1763; Davies, M.R., *et al.*, 2003. J Am Soc Nephrol 14:1559-1567; Davies, M.R., *et al.*, 2005. J Am Soc Nephrol 16:917-928; Lund, R.J., *et al.*, 2004. J Am Soc Nephrol 15:359-369; Mathew, S., *et al.*, 2008. J Am Soc Nephrol 19:1092-1105. PMCID:PMC2396927; Mathew, S., *et al.*, 2007. J Am Soc Nephrol 18:122-130; Mathew, S., *et al.*, 2008. J Am Soc Nephrol 19:1509-1519. PMCID:PMC2488263.). Kidney function measured by inulin clearance and by BUN levels was reduced to the level of human stage 3 CKD in *ldlr-/-* high fat-fed mice, as described in Section 9.4.1.1, and these mice are hereafter referred to as CKD-3 (Fig. 12A and Fig. 12B). Furthermore, the CKD-3 mice were hyperphosphatemic (Table 11) which is consistent with human stage 3b-4 CKD. A second model of CKD was the spontaneous CKD that develops in mice and humans deficient in the α5 chain of type IV collagen (Col4α5) (Alport's syndrome) (Rheault, M.N., *et al.*, 2004. Journal of the American Society of Nephrology 15:1466-1474.). Col4α5-deficient mice at 200 days of age have reductions in inulin clearance and BUN elevations equivalent to human stage 3-4 CKD (Fig. 12C and Fig. 12D). A third model, CKD-3 in the GNZ mouse is described in Section 9.4.1.1 and below.

[00484] **Table 11.** Serum biochemical parameters in the various groups of animals.

Parameter	**Group 1 Wild type**	**Group 2 Sham**	**Group 3 CKD-3**	**Group 4 CKD-3**	**Group 5 CKD-3**
Strain	C57/BJ6	*Ldlr -/-*	*Ldlr -/-*	*Ldlr -/-*	*Ldlr -/-*
Diet	Chow	High fat	High fat	High fat	High fat
Surgery	None	Sham	CKD	CKD	CKD
Weeks postnatal	28	28	28	28	28
Treatment	None	None	Vehicle	mActRIIA-Fc	LaC03
N	12	15	14	15	12
BUN (mg/dL)	24.0 ± 4.6	20.6 ± 3.7	37.7 ± 7.6	36.5 ± 5.8	35.6 ± 9.1
Ca (mg/di)	8.3 ± 1.8	8.9 ± 0.9	9.4 ± 0.8	8.8 ± 0.3	8.8 ± 1.4
Phosphorous (mg/di)	8.9 ± 0.2	7.9 ± 2.3	11.0 ± 1.6	11.8 ± 1.2	10.5 ± 2.1

9.4.2.2 Activin levels and Aortic Activin Receptor Type IIA (ActRIIA)

[00485] The ability of renal repair in CKD to stimulate circulating levels of a TGFβ superfamily member was investigated. Circulating activin levels were increased in models of CKD (Fig. 13A and Fig. 13B). Kidney and aortic tissues were analyzed for the mRNA for activin A (a homodimer of Inhibin betaA (Inhba)). Inhba mRNA was induced by CKD in the kidney and aorta (Fig. 13C), and protein levels were increased in the kidney (Fig. 13D). Aortas were analyzed from mice with CKD-3 for induction of TGFβ superfamily type II receptors, which are the ligand binding component of the superfamily receptor heteromultimers composed of type II and type I (ALK) receptors. CKD-3 produced in the *ldlr-/-* high fat-fed mouse induced up regulation of the Activin type II receptor A (ActRIIA) in the aortic vascular smooth muscle (Fig. 13E). Activins associate with regulated inhibitory factors, follistatin and follistatin like 3 (fstL3) (Welt, C., *et al.*, 2002. Experimental Biology and Medicine 227:724-752.), whose circulating levels (Fig. 13F and Fig. 13G) and tissue levels were not affected by CKD-3. The stoichiometry of follistatin, fstL3, and inhibin (sum of 620pg/ml plus 400pg/ml of unmeasured inhibin (Sharpe, R.M., *et al.*, 1999. Journal of Andrology 20:94-101.)) to Activin A levels (>5000pg/ml) in the circulation suggests that CKD produces significant free activin levels, a pathologic event making activin A a circulating factor in CKD.

9.4.2.3 Aortic endothelial-mesenchymal transition in CKD

[00486] A third experimental model of CKD was utilized for these studies. The GNZ mouse is used for cellular lineage tracing (Stoller, J.Z., *et al.*, 2008. *Genesis (New York, N.Y. : 2000)* 46:200-204.), and GNZ mice express nuclear-localized Green Fluorescent Protein and beta galactosidase (GFP/LacZ) once an upstream *loxP* flanked STOP sequence is removed (Fig. 14A). To remove the *loxP* sites in the GNZ mice, they were bred with Tek-Cre mice expressing Cre recombinase under the direction of the tyrosine kinase Tek (Tie2) promoter/enhancer. Since Tie2 is an endothelial lineage specific angiopoietin receptor, the GNZ/Tek-Cre+ mice express nuclear and cytoplasmic GFP in endothelial lineage cells (Fig. 14A) (Moore-Morris, T., *et al.*, 2014. The Journal of Clinical Investigation 124:2921-2934.). In GNZ/Tek-Cre+ mice, aortic GFP co-localized with the nuclear DAPI stain is limited to endothelial cells marked with the CD31 biomarker (Fig. 14B, *see* arrows). When CKD-3 was produced in GNZ/Tek-Cre+ mice, in addition to endothelial cells, cells in the aortic media and adventia demonstrated GFP staining, showing that these cells derived from an endothelial lineage through endothelial-mesenchymal transition (EnMT) (Fig. 14C, 14D, *see* arrows). To further analyze whether CKD stimulates

EnMT, the expression of a transcription factor involved in EnMT, Snai 1, was examined in a model of spontaneous CKD, the Alports's syndrome mouse. Snai 1 was upregulated early (at 75 doa) in the course of CKD (Fig. 14E), consistent with the timing of peak Activin levels (*see* below).

9.4.2.4 Vascular effects of inhibiting ActRIIA signaling in CKD

[00487] A ligand trap (Fig. 23A) comprised of the extracellular domain of ActRIIA fused to the Fc domain of IgG1, (herein referred to as mActRIIA-Fc) was utilized. Figure 23B depicts the experimental design for utilization of mActRIIA-Fc in a vascular calcification model using a treatment protocol which allowed vascular calcification stimulated by CKD-3 to develop for eight weeks prior to instituting mActRIIA-Fc. Using this protocol, treatment of CKD-3 mice with the ActRIIA ligand trap decreased CKD-3 stimulated aortic Runx2 expression and aortic alkaline phosphatase (ALP) expression in the *ldlr-/-* high fat fed CKD-3 mice (Fig. 25A). Both Runx2 and ALP expression represent potential downstream effects of ActRIIA signaling and biomarkers of osteoblastic transition in the aorta that was reversed by mActRIIA-Fc-treatment. Aortic smooth muscle 22α (Sm22-alpha), a biomarker of differentiated vascular smooth muscle cells was decreased by CKD-3 and stimulated by mActRIIA-Fc. CKD-3 caused decreased aortic myocardin (MYOCD) expression, the vascular smooth muscle cell specific transcription factor, but myocardin was not affected by mActRIIA-Fc-treatment. Aortic Klotho expression, which has been linked to vascular calcification (Lim, K., *et al.*, 2012. Circulation 125:2243-2255.), was very low compared to kidney (data not shown). In terms of the effects of CKD-3 and mActRIIA-Fc-treatment on aortic protein levels of the mRNAs in Figure 25A, CKD increased aortic Runx2 levels and mActRIIA-Fc normalized aortic Runx2 levels (Fig. 25B). CKD decreased the aortic levels of alpha smooth muscle actin (αSMA), another biomarker of differentiated vascular smooth muscle cells, and mActRIIA-Fc treatment increased aortic levels of αSMA (Fig. 25B). Myocardin levels were not changed by CKD- or mActRIIA-Fc-treatment.

9.4.2.5 Effects of decreased ActRIIA signaling on vascular calcification

[00488] CKD stimulation of vascular calcification was studied in a model of atherosclerosis and type 2 diabetes (Al-Aly, Z., *et al.*, 2007. Arteriosclerosis, Thrombosis, and Vascular Biology 27:2589-2596; Towler, D.A., *et al.*, 1998. Journal of Biological Chemistry 273:30427-30434.), the *ldlr-/-* high fat-fed mouse with ablative CKD, as described above. CKD-3 caused accumulation of calcium deposits in the aortic atheromas in CKD-3 vehicle treated mice (CKD-3 V) (Fig. 15A), which were not present in CKD-3 mice treated with mActRIIA-Fc

(CKD-3 R), and the aortic tissue calcium content was reduced to levels observed in wild type mice, significantly below those present at the time of institution of mActRIIA-Fc treatment, CKD-3 (Fig. 15B).

9.4.2.6 Effects of decreased ActRIIA signaling on CKD stimulated cardiac disease

[00489] CKD stimulation of cardiac disease was studied in the vascular calcification model. CKD-3 V mice had increased heart weight which was reversed by mActRIIA-Fc-treatment (Fig. 16A), but not by lanthanum carbonate treatment (CKD-3 L (Fang, Y., *et al.*, 2014. J Am Soc Nephrol 25:1760-1763.)), and CKD-3 V induced mild cardiac hypertrophy (Fig. 16B). However, while there was no evidence of cardiac fibrosis (Fig. 16C), there was evidence of myocyte hypertrophy, which was reversed by mActRIIA-Fc-treatment.

[00490] Since one mechanism of vascular calcification and cardiac hypertrophy association in CKD is through large artery stiffness, vascular stiffness and blood pressures were measured using previously reported methods (Wagenseil, J.E., *et al.*, 2009. *Circulation Research* 104:1217-1224; Wagenseil, J.E., *et al.*, 2005. *AJP - Heart and Circulatory Physiology* 289:H1209-H1217.). CKD-3 in the *ldlr-/-* high fat-fed atherosclerotic mouse did not produce vascular stiffness of carotid arteries or of the aorta (Figs. 19A and 19B) or blood pressure elevations (Table 12). Furthermore, Dkk1 neutralization in CKD-3 mice treated with neutralizing antibody to Dkk1 (Fang, Y., *et al.*, 2014. J Am Soc Nephrol 25:1760-1763.) had no effect on aortic stiffness in the absence of increased stiffness.

[00491] **Table 12.** Physiologic cardiovascular parameters of 28 weeks old mice.

	WT	Sham	CKD-3 V	CKD-3 Dkk1 mAb
SBP	108.9±1.1	111.9±4.7	100.4±4.6	110.7±4.8
DBP	77.27±1.0	78.56±2.2	69.14±3.1	77.55±3.6
Pulse Pressure	31.63±0.3	33.32±2.5	31.27±2.1	33.1±1.9
Heart Rate	547.1±4.7	501.2±17.4	505.6±14.3	558.6±11.2

SBP=systolic blood pressure, DBP=diastolic blood pressure. Values are presented as mean ± standard error of the mean. N=4-6 mice per group.

9.4.2.7 ActRIIA signaling in aorta and kidney and kidney fibrosis

[00492] Canonical signal transduction by the TGFβ superfamily involves ligand binding to type II receptors activating their serine/threonine kinase activity and stimulating association and phosphorylation of type I receptors, the Alk kinases (*see* diagrammatic representation in Fig. 20). There are seven Alk kinases utilized by the TGFβ superfamily, and Alk4 (ActRIB) is the type I receptor most often associated with activin/ActRIIA signaling (Abe Y, *et al.*, 2004.

Growth Factors (Chur, Switzerland) 22:105-110.). In kidney homogenates from the CKD-3 mouse model, Alk4 phosphorlyation was not increased, in agreement with previous studies (Antsiferova, M., and Werner, S. 2012. Journal of Cell Science 125:3929-3937.) (Fig. 18A). In contrast, phosphosmad 2 was increased by CKD -3 and was decreased by mActRIIA-Fc (Fig. 18A), indicating a role of another Alk receptor in renal activin signaling in CKD. In the diseased CKD-3 kidney with interstitial nephritis, Col1A1 was upregulated and mActRIIA-Fc decreased Col1a1 levels (Fig. 18A), consistent with the decrease in phosphosmad 2 induced by mActRIIA-Fc. Furthermore, there was a major decrease in renal Klotho expression induced in the CKD-3 V mice that was corrected by mActRIIA-Fc (Fig. 18B). As a result, the renal compartments where Activin was expressed in CKD-3 V were analyzed and heavy expression in peritubular myofibroblasts, but not in the epithelium, was identified.

[00493] In the aortic smooth muscle, Alk4 and Alk1 (Fig. 18A), but not Alk5 or Alk2, were detected. The levels of Alk4 and Alk1 were not affected by CKD-3. However, CKD-3 did not increase aortic phosphosmad 2/3 levels, nor did mActRIIA-Fc decrease them (Fig. 18A). However, analysis of noncanonical ActRIIA signaling (Fig. 20) indicated that map kinase (phosphoErk1/2) was not activated by CKD nor inhibited by mActRIIA-Fc and that vascular smooth muscle levels of p38 and JNK were very low. Aortic Actin2 levels were also very low, and were not CKD-3-stimulated. Axin2 levels are a standard biomarker of canonical Wnt signaling. However, since Dkk1 is a transcriptional target of canonical Wnt signaling, Dkk1 levels in the kidney and aorta were analyzed as a biomarker of Wnt activity, demonstrating that mActRIIA-Fc-treatment decreased renal Dkk1 levels in the CKD-3 mice (Fig. 18C), producing significant reductions in plasma Dkk1 levels (Fig. 18C). Furthermore, aortic Dkk1 levels were increased by CKD-3 and inhibited by mActRIIA-Fc (Fig. 18C). This indicated that renal and vascular smooth muscle Wnt signaling and systemic release of Dkk1 were inhibited by activin suppression. Accordingly, elevated circulating activin in CKD signals through increased vascular ActRIIA activating Wnt signaling and stimulating atherosclerotic calcification.

9.4.2.8 Translation of preclinical studies to human pathophysiology and the clinic

[00494] As shown in Fig. 19, serum activin levels were increased in a cohort of patients with CKD stage 3, as compared to healthy controls.

9.4.3 CONCLUSIONS

[00495] This example demonstrates that activin is a second factor involved in renal repair that is increased in the circulation during CKD. Without being bound by theory, the results demonstrate that Activin expression is increased in diseased kidneys, is released into the circulation and stimulates aortic vascular smooth muscle dedifferentiation and osteoblastic transition, which is contributed to by endothelial to mesenchymal transition and these effects culminate in CKD-stimulated atherosclerotic calcification and cardiac hypertrophy. CKD upregulates renal Activin expression in myofibroblasts and stimulates progression of kidney disease through increasing renal fibrosis. Inhibition of ActRIIA signaling inhibits osteoblastic transition and atherosclerotic calcification in the aorta. Inhibition of ActRIIA signaling inhibits cardiac hypertrophy and renal fibrosis. The effects of the ActRIIA ligand trap, mActRIIA-Fc, on the early CKD-MBD syndrome resemble those of a monoclonal antibody to Dkk1 in CKD-stimulated vascular calcification (Fang, Y., *et al.*, 2014. J Am Soc Nephrol 25:1760-1763.). These data demonstrate that inhibition of ActRIIA signaling in the diseased kidney decreased Wnt activation and circulating Wnt inhibitors. Furthermore, in the vascular smooth muscle, activin stimulates Wnt activity, which is inhibited by mActRIIA-Fc. Thus, reactivation of two kidney development factors, Wnts and Activin, produce circulating Wnt inhibitors and activin in CKD, causing vascular and cardiac disease. This is the demonstration that kidney diseases directly cause cardiovascular disease and that consideration of the kidney disease process cannot be separated from renal repair and the consequent stimulation of cardiovascular disease.

[00496] Further, this example demonstrates that renal activin expression is increased in three models of chronic kidney disease, producing increased circulating activin that exceeds the levels of activin inhibitors in the circulation. Circulating Activin stimulated signal transduction through the ActRIIA receptor producing aortic vascular smooth muscle dedifferentiation, osteoblastic transition, and calcification of atherosclerotic plaques. These effects were due to circulating activin, as supported by the absence of increased activin protein levels in the aorta even though CKD induced increased aortic activin mRNA (Fig. 13B). Without being bound by theory, these data demonstrate that vascular activin signaling is stimulated by CKD through the production of endocrine activin by the kidney.

[00497] Since EnMT is a Smad-mediated process (Cooley, B.C., *et al.*, 2014. Science Translational Medicine 6:227ra234.), activated by members of the TGFβ superfamily that includes TGFβs, bone morphogenetic proteins (BMPs), activins/inhibins, and growth and

differentiation factors (GDFs) (Piek, E., *et al.*, 1999. The FASEB Journal 13:2105-2124.), the aortas of the vascular calcification model with CKD-3 were analyzed for changes in the levels of type II receptors of the TGFβ superfamily, which are the ligand binding components of the receptors. The activin receptor type IIA (ActRIIA) was upregulated in aortic vascular smooth muscle. Administration of the extracellular domain of ActRIIA in a fusion protein with the Fc domain of IgG1 as a ligand trap (mActRIIA-Fc) demonstrated that inhibition of ActRIIA signaling produced inhibition of osteoblastic transition in the aortic smooth muscle cells, stimulation of vascular smooth muscle cell gene expression and reversal of aortic calcification in a CKD stimulated atherosclerotic model. In addition, CKD stimulated EnMT in a lineage tracing mouse model. However, the major site of activin function appeared to be in the vascular smooth muscle, where ActRIIA expression was increased.

[00498] Cardiac hypertrophy, especially left ventricular, is highly prevalent in CKD (Moran, A., *et al.*, 2008. American Journal of Kidney Diseases 52:839-848; Park, M., *et al.*, 2012. Journal of the American Society of Nephrology 23:1725-1734.). In addition, the atherosclerotic vascular calcification model was characterized by cardiac hypertrophy (Fig. 16). However, production of cardiac hypertrophy in the model differs mechanistically from the common paradigm of CKD-stimulated vascular stiffness (Townsend, R.R. 2015. Current Opinion in Nephrology and Hypertension 24:47-53 10.1097/MNH.0000000000000086; Merx, M.W., *et al.*, 2005. Journal of the American Society of Nephrology 16:3357-3364.), hypertension, and cardiac remodeling, because the atherosclerotic CKD-3 mice (*ldlr-/-* high fat-fed diabetic mice) did not exhibit vascular stiffness nor hypertension. Since mActRIIA-Fc corrected the cardiac hypertrophy in the absence of changes in FGF23 levels (Fig. 19), it is unlikely that the cardiac hypertrophy in the CKD-3 mice was due to FGF23 (Faul, C., *et al.*, 2011. *J Clin Invest* 121:4393-4408.).

[00499] In conclusion, without being bound by theory, this example demonstrates that a second factor involved in renal repair that is increased in the circulation during CKD and causes cardiovascular disease. Without being bound by theory, the example demonstrates that Activin expression is increased in diseased kidneys, is released into the circulation and stimulates aortic vascular smooth muscle dedifferentiation and osteoblastic transition, which is contributed to by endothelial to mesenchymal transition and these effects culminate in CKD-stimulated atherosclerotic calcification, and cardiac hypertrophy. CKD upregulates renal Activin expression in myofibroblasts and stimulates progression of kidney disease through increasing

renal fibrosis. Inhibition of ActRIIA signaling inhibits osteoblastic transition and atherosclerotic calcification in the aorta. Inhibition of ActRIIA signaling inhibits cardiac hypertrophy and renal fibrosis. The effects of the ActRIIA ligand trap, mActRIIA-Fc, on the early CKD-MBD syndrome show that inhibition of ActRIIA signaling in the diseased kidney decreased Wnt activation and circulating Wnt inhibitors. Thus, without being bound by theory, reactivation of two kidney development factors, Wnts and Activin, produce circulating Wnt inhibitors and activin in CKD, causing vascular and cardiac disease; thus, kidney disease causes cardiovascular disease.

9.5 EXAMPLE 5: KIDNEY INJURY/REPAIR STIMULATES VASCULAR DISEASE THROUGH SYSTEMIC WNT INHIBITION AND ACTIVIN

9.5.1 BACKGROUND

[00500] The results presented in this example relate to the results presented in Example 4 (Section 9.4).

[00501] Kidney diseases cause atherosclerotic vascular calcification by producing systemic Wnt inhibition and activin secretion during kidney repair. Vascular effects of chronic kidney disease (CKD) are an interplay of Wnt inhibition and activin-induced modulation of activin receptor function.

9.5.2 METHODS

[00502] CKD with elevated Wnt inhibitors, especially Dkk1, and activin was induced in mouse models for lineage tracing and vascular calcification and allowed to develop spontaneously in Alport's mice. Activin, Dkk1, activin receptor type 2A (ActRIIA), phosphosmad 2/3, and collagen levels were measured by ELISA, RT-PCR, and western blots. Vascular smooth muscle function was measured by pressure-induced arterial dilatation. Cell lineage tracing was performed in Rosa-tdT mice bred to endothelial-specific Tie2-Cre mice. Mice harboring Rosa-tdT express tomato red in cells harboring Cre recombinase. mActRIIA-Fc refers to an activin A ligand trap (*see*, for example, U.S. Patent No. 8,173,601 and Carrancio *et al.*, 2014, British Journal of Haematology, 165:870-882).

9.5.3 RESULTS

[00503] CKD increased circulating Dkk1 and activin levels. In diseased kidneys, activin was expressed in myofibroblasts, and ActRIIA signaling through phosphosmad 3 was increased.

In diseased kidneys, mActRIIA-Fc, an ActRIIA ligand trap, inhibited renal psmad 3, Col1A1 expression, urinary protein levels, and Dkk1 levels and increased Klotho levels. In vasculature, ActRIIA levels and signaling were impaired by CKD along with decreased vascular smooth muscle differentiation and function. In CKD mice vasculature, mActRIIA-Fc increased vascular smooth muscle cell differentiation and inhibited osteoblastic transition and vascular calcification. In circulation, mActRIIA-Fc decreased Dkk1 levels. CKD induced expression of tomato red in cells of the adventia of injured femoral arteries of Tek-Cre/Rosa-tdT CKD mice versus Tek-Cre/Rosa-tdT mice with normal kidney function, wherein the tomato red was limited to femoral artery endothelium, demonstrating that CKD induces Tie2-positive cells during vascular injury.

9.5.4 CONCLUSIONS

[00504] CKD decreased vascular smooth muscle function and stimulated osteoblastic transition and vascular calcification. Decreasing effects of elevated activin in CKD with mActRIIA-Fc inhibited Smad-dependent renal fibrosis, blocked aortic osteoblastic transition, increased vascular smooth muscle differentiation, and decreased vascular calcification.

9.6 EXAMPLE 6: THE ROLE OF ACTIVIN SIGNALING IN THE PATHOGENESIS OF RENAL OSTEODYSTROPHY OF THE CKD-MBD

9.6.1.1 INTRODUCTION

[00505] Chronic kidney disease-mineral/bone disorder (CKD-MBD) includes vascular calcification and osteodystrophy. CKD increases circulating activin (a ligand of the TGFβ superfamily) and activin signaling (Figure 21A). Inhibition of ActRIIA signaling with an activin type IIA receptor (ActRIIA) ligand trap (mActRIIA-Fc) inhibits vascular calcification and prevents cardiac hypertrophy. This example demonstrates the role of activin signaling in the pathogenesis of renal osteodystrophy.

9.6.1.2 METHODS

[00506] Sham operated ldlr-/- high-fat fed mice (SHAM; n=12) manifest diabetes and hypercholesterolemia. CKD with hyperphosphatemia, elevated FGF-23, and 60% reduction in glomerular filtration rate (CKD-3) was induced by 5/6 nephrectomy at 14 weeks of age in the ldlr-/- high-fat-fed mice, and is a model of atherosclerotic vascular calcification. CKD-3 mice were treated with mActRIIA-Fc 10 mg/kg (mActRIIA-Fc; n=15) or vehicle (VEH; n=13), injected intraperitoneally weekly beginning at 22 weeks of age and studied at 28 weeks by

skeletal histomorphometry and micro-computed tomography. Results of CKD-3 were compared with wild type (WT; n=5) mice and SHAM.

9.6.2 RESULTS

[00507] Relative to WT (cancellous bone volume/tissue volume (BV/TV): 12.90%), SHAM mice demonstrated reduced BV/TV (10.92%) associated with adynamic bone disease. Induction of CKD-3 caused high turnover bone disease in VEH mice, and a lower BV/TV (11.22%); this was reversed by 6 weeks of mActRIIA-Fc-treatment (13.28%). Induction of CKD-3 caused a reduction in trabecular thickness, which was reversed by 6 weeks of mActRIIA-Fc-treatment. CKD-3 VEH-treated mice demonstrated higher erosion surface/bone surface and higher osteoclast number/100 mm bone length (1.83% and 62.32/100 mm, respectively) compared with SHAM (1.05% and 33.40/100 mm), which were mitigated by mActRIIA-Fc (1.23% and 38.37/100 mm). CKD-3 VEH-treated mice also caused demonstrated higher osteoblast surface/bone surface and higher osteoblast number/100 mm bone length (1.58% and 110.63/100 mm respectively; $P<0.05$) when compared with WT or SHAM (Figure 21B and Figure 21C, respectively). mActRIIA-Fc significantly reduced both osteoblast surface/bone surface and osteoblast number/100 mm bone length (0.68% and 43.18/100 mm; $P<0.05$) compared with VEH. Despite the significant reduction in the osteoblast number relative to VEH, the mineral apposition rate with mActRIIA-Fc treatment was maintained (0.42 and 0.40 μm3/day, respectively), with a significantly higher bone formation rate/osteoblast (0.17 vs. 0.48 μm3/100 cells/year, respectively; $P<0.05$ vs. VEH), which was similar to WT (0.42 μm3/100 cells/year) (Figure 21D).

[00508] **Table 13.** Histomorphometric Results (mean + SEM)

	Sham	**CKD-3 + Vehicle**	**CKD-3 + mActRIIA-Fc**
BV, %	10.9±1.3	11.2±0.8	13.3±1.2
Trabecular thickness (plate), µm	30.9±2.1	31.8±1.7	33.2±1.9
Trabecular separation (plate) µm	269.4±17.3	261.9±16.6	238.1±24.9
Osteoid volume/BV, %	0.3±0.1	0.4±0.1	0.2±0.1
Osteoid surface/bone surface, %	2.5±0.6	2.9±0.7	1.7±0.4
Osteoid thickness, µm	2.1±0.4	1.9±0.3	1.9±0.2
Erosion surface/bone surface, %	1.1±0.2	1.8±0.5	1.2±0.5
Osteoclast number/bone perimeter, #/100 mm	33.4±5.1	62.3±19.5	38.4±15.4
Osteoclast surface/bone surface, %	1.0±0.2	1.7±0.5	1.1±0.5
Mineral apposition rate/day, µm/daty	0.4±0.0	0.4±0.1	0.4±0.0
Double labels/bone surface, %	3.0±0.9	3.7±1.0	1.6±0.3
Single labels/bone surface, %	8.0±0.9	10.1±1.1	10.3±1.9
Mineralizing surface/bone surface, %	7.0±0.9	8.8±1.1	6.8±1.1
Bone formation rate/bone surface, mm^3/cm^2/year	10.8±2.3	13.9±2.5	9.5±1.2
Mineralization lag time, days	1.9±0.4	1.9±0.5	1.3±0.3
Osteoid maturation time, days	6.3±1.4	5.4±0.9	4.9±0.5

SEM= standard error of the mean

[00509] mActRIIA-Fc did not affect hyperphosphatemia and FGF-23 levels.

9.6.3 CONCLUSIONS

[00510] Increased circulating activin contributes to the high turnover osteodystrophy associated with CKD-3. Activin signaling inhibition with mActRIIA-Fc, an ActRIIA ligand trap, increased bone volume in CKD-3 by inhibiting bone resorption and normalizing the mineral apposition rate and bone formation rate/osteoblast, counteracting the negative effects of CKD.

9.7 EXAMPLE 7: QUANTITATIVE COMPUTED TOMOGRAPHY RESULTS FOR BONE MASS AND ABDOMINAL AORTIC VASCULAR CALCIFICATION IN HEMODIALYSIS SUBJECTS TREATED WITH ESCALATING DOSE LEVELS OF hACTRIIA-Fc

9.7.1 INTRODUCTION

[00511] High turnover renal osteodystrophy is marked by decreased cortical and increased trabecular bone mass, resulting in an increased risk of fracture. hActRIIA-Fc, an activin type II receptor-IgG1 fusion protein, blocks activin A signaling and may reduce osteoclastogenesis and promote osteoblast maturation in bone, based on in vitro data. The current analysis in subjects on hemodialysis (HD) evaluated the effect of hActRIIA-Fc on bone mineral density (BMD) and abdominal aortic vascular calcification using quantitative computed tomography (QCT).

9.7.2 METHODS

[00512] In a study of hActRIIA-Fc in subjects on HD for the correction of anemia, subjects who were erythropoietin-stimulating agent (ESA) responsive were washed out of their ESA effect until hemoglobin (Hb) was <10 g/dL, then randomized to hActRIIA-Fc 0.3 mg/kg (n=9), 0.5 mg/kg (n=8), 0.7 mg/kg (n=9; 7 completed, 2 ongoing), or placebo (PBO; n=9) administered subcutaneously every 28 days for up to 8 dose cycles. A dose group utilizing a 14-day dose cycle is currently enrolling. Subjects were assessed for effects on Hb, safety parameters, BMD, vascular calcification, and biomarkers of bone turnover. Treatment failures (Hb <9 g/dL) were rescued with ESA/transfusion. QCT scans of the hip, lumbar spine, and abdominal aorta were obtained at baseline and after the 225-day treatment phase.

9.7.3 RESULTS

[00513] Of the 35 subjects who were randomized in the study, 20 had paired QCT assessments (PBO: n=3; 0.3 mg/kg: n=6; 0.5 mg/kg: n=5; and 0.7 mg/kg: n=6). Some subjects with paired QCT had limited exposure to hActRIIA-Fc due to treatment failure. Table 14 displays the BMD and vascular calcification results for PBO and each hActRIIA-Fc dose group. hActRIIA-Fc appeared to mitigate the effects of high turnover renal osteodystrophy on cortical and trabecular BMD and slow the progression of vascular calcification, compared with PBO.

[00514] Table 14

			hActRIIA-Fc					
	PBO	n	0.3 mg/kg	n	0.5 mg/kg	n	0.7 mg/kg	n
Days on study drug	107.3	3	107.2	6	152.6	5	151.8	6
Femoral neck cortical BMD, mean % CFB	-0.91%	3	-1.38	5	1.56%	5	0.90%	4
Subjects with >2% increase in femoral neck cortical BMD, %	0.00%	3	20.00%	5	40.00%	5	75.00%	4
Lumbar BMD (trabecular), mean % CFB	12.59%	3	7.95%	6	0.54%	5	1.93%	6
Total Agatston score, mean % CFB	58.42%	3	24.89%	6	17.26%	5	7.42%	5
Subjects with <15% increase in Agatston score, %	33.33%	3	83.33%	6	80.00%	5	100.00%	5
Square root of volumetric score (mm^3), mean CFB	4.54	3	11.07	6	1.23	5	1.26	5

CFB=change from baseline.

9.7.4 CONCLUSIONS

[00515] These data demonstrate that hActRIIA-Fc, in a dose-dependent manner, reverses the effects of high turnover renal osteodystrophy on cortical and trabecular bone, and slows the progression of vascular calcification.

9.8 EXAMPLE 8. QUANTITATIVE COMPUTED TOMOGRAPHY RESULTS FOR BONE MASS AND ABDOMINAL AORTIC VASCULAR CALCIFICATION IN HEMODIALYSIS SUBJECTS TREATED WITH ESCALATING DOSE LEVELS OF ACTRIIA-HFC (SEQ ID NO:7; "SOTATERCEPT"):

9.8.1 INTRODUCTION

[00516] *See* the Introduction (Section 9.7.1) and Methods (Section 9.7.2) for Example 6 (Section 9.7). This example presents additional data from the study performed in Example 6 (Section 9.7), obtained at a later date in the study.

9.8.2 RESULTS

[00517] A total of 35 subjects were randomized and received at least one dose of placebo or ActRIIA-hFc (SEQ ID NO:7; "Sotatercept") as indicated in Table 15.

[00518] **Table 15.** Randomized Subjects and QCT Analysis Subset

Subjects	Placebo	Sotatercept 0.3 mg/kg	Sotatercept 0.5 mg/kg	Sotatercept 0.7 mg/kg
Randomized and received ≥ 1 dose of placebo or Sotatercept	9	9	8	9
QCT measures at baseline and Day 225	4	6	5	6

[00519] The subject disposition is depicted in Figure 22A, including the subjects with paired QCT measurements at baseline and day 225. Most subjects who were discontinued from study treatment had treatment failure requiring rescue, generally because hemoglobin concentration was below 9 g/dL. Most subjects who were discontinued from study treatment received placebo or 0.3 mg/kg of sotatercept. None of the subjects discontinued treatment because of an adverse event. Further, of the 21 subjects with paired QCT measurements, 12 required rescue therapy due to hemoglobin treatment failure during the treatment phase, 9 of whom required rescue within the first 3 dose cycles.

[00520] Among subjects with paired QCT measurements, baseline demographic and clinical characteristics were generally similar across treatment groups (Table 16). However, there was a substantially longer time on dialysis in the placebo group, which was also the youngest group. There were also differences between groups in baseline biomarker and Agatston scores (Table 17).

[00521] **Table 16.** Baseline demographic and clinical characteristics of subjects with paired QCT measurements

	Placebo (n=4)	**Sotatercept 0.3 mg/kg (n=6)**	**Sotatercept 0.5 mg/kg (n=5)**	**Sotatercept 0.7 mg/kg (n=6)**
Age, mean, years	51.3	57.3	60.8	63.0
Female, n (%)	1 (25.0)	6 (66.7)	0 (0.0)	4 (66.7)
Race, n (%)				
White	1 (25.0)	3 (50.0)	3 (60.0)	5 (83.3)
Black	2 (50.0)	3 (50.0)	2 (40.0)	1 (16.7)
Asian	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ethnicity, n (%)				
Hispanic	0 (0.0)	2 (33.3)	3 (60.0)	2 (33.3)
Non-Hispanic	4 (100.0)	4 (66.7)	2 (40.0)	4 (66.7)
Postdialysis weight, mean, kg	65.5	80.3	79.4	84.5
Body mass index, mean, kg/m^2	24.2	27.7	26.9	29.9
Diabetes, n (%)	2 (50.0)	5 (83.3)	5 (100.0)	5 (83.3)
Parathyroidectomy, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Time on dialysis, mean, months	165.8	43.3	22.4	68.1
Non-calcium phosphate binder, n (%)*	4 (100.0)	3 (50.0)	3 (60.0)	3 (50.0)
Calcium-based phosphate binder, n (%)*	1 (25.0)	4 (66.7)	4 (80.0)	4 (66.7)
Calcimimetic, n (%)*	1 (25.0)	2 (33.3)	0 (0.0)	2 (33.3)
1,25-OH vitamin D analog, n (%)	3 (75.0)	5 (83.3)	2 (40.0)	4 (66.7)

* subjects could be receiving multiple types of binders

[00522] **Table 17.** Mean baseline biomarker, volumetric BMD, and Agatston scores of subjects with paired QCT measurements

	Placebo (n=4)	**Sotatercept 0.3 mg/kg (n=6)**	**Sotatercept 0.5 mg/kg (n=5)**	**Sotatercept 0.7 mg/kg (n=6)**
Baseline whole PTH, pg/mL	209.2	104.8	135.5	100.4
BSAP[a], µg/L	24.0	18.0	13.2	15.5
P1NP[b], ng/mL	548.3	376.2	468.4	437.5
CTX[c], pg/mL	3,152.3	2,062.5	2,266.8	2,246.5
Total hip integral BMD, mg/cm^3	276.8	286.6	280.0	281.0
Femoral neck cortical BMD, mg/cm^3	640.5	667.0	593.1	594.8
Mean spine (L1, L2) BMD, mg/cm^3	140.1	125.6	149.1	118.7
VC total Agatston score[d]	6,498.8	9,472.7	3,618.5	1,862.1

Note: The n reflects the number of randomized subjects with paired QCT assessments; the actual number of subjects available for each parameter may vary. PTH=parathyroid hormone.
[a]Bone=specific alkaline phosphatase (BSAP) reference range: males, 6-30; females (premenopausal), 3-19; females (postmenopausal), 6-26.
[b]Procollagen type 1 N-propeptide (P1NP) reference range: males, 30-110; females 20-108
[c]C-terminal telopeptide (CTX) reference range: males, 0-854; females (premenopausal), 26-573; females (postmenopausal), 104-1,008
[d]Lower Agatston scores indicate lower levels of vascular calcification.

[00523] Serious adverse events were generally considered unrelated to sotatercept, did not lead to discontinuation, and resolved with continued therapy. No deaths were reported in the sotatercept treatment groups. Adverse events were mostly mild or moderate in severity, unrelated to the study drug, relatively similar between treatment groups, and generally consistent with the subjects' medical histories. In the first 28-day dose cycle and during the 225-day treatment phase, home blood pressure measurements showed no consistent or dose-dependent changes from baseline among subjects in any of the treatment groups.

[00524] The changes from baseline in abdominal aorta total Agatston scores are provided in Table 18. The proportion of subjects with < 15% progression in abdominal aorta total Agatston scores is shown in Figure 22B.

[00525] **Table 18.** Baseline and change from baseline in abdominal aorta total Agatston score and square root transformed total volume score*

	Placebo (n=4)	Sotatercept 0.3 mg/kg (n=6)	Sotatercept 0.5 mg/kg (n=5)	Sotatercept 0.7 mg/kg (n=6)
Abdominal aorta total Agatston score				
Baseline total Agatston score	6,498.8	9,472.7	3,618.5	1,862.1
Change from baseline total Agatston score	787.8	8.578.9	225.7	171.0
% change from baseline total Agatston score	58.4	29.9	17.3	7.4
Square root transformed total volume score				
Baseline square root of total volume, mm^3	39.0	46.2	33.1	27.4
Change from baseline square root of total volume, mm^3	3.4	11.1	1.2	1.3

Note: The n reflects the number of randomized subjects with paired QCT assessments; actual number of subjects available for each parameter may vary.
*Lower total Agatston and square root transformed total volume scores indicate lower levels of vascular calcification.

[00526] Further, Table 19 provides the percent change from baseline in femoral neck cortical and mean lumbar spine trabecular volumetric BMD measurements. The proportions of subjects with >2% gain in femoral neck cortical BMD were analyzed and it was determined that treatment with sotatercept is associated with increases in the proportion of subjects with ≥2% increase in femoral neck cortical bone (*see* Table 19 and Figure 22C). *See, also,* Malluche *et al.*, 2014, Clin. J. Am. Soc. Nephrol., 9:1254-1262. Moreover, in high-turnover ROD, trabecular bone mass increases, as measured by lumbar spine trabecular BMD, without a reduction in vertebral fracture rates in ESKD as compared with the general population (*see*, Leonard MB, 2009, Semin. Nephrol., 29:133-143, and Duan *et al.*, 1999, J. Clin. Endocrinol. Metab. 84:718-722). In this setting, the increased trabecular bone mass is of poor quality (*see* Malluche *et al*, 2012, J. Am. Soc. Nephrol. 23:525-532. Treatment with sotatercept slows the increase in trabecular bone mass in the lumbar spine (Table 19).

[00527] **Table 19.** Baseline and percent change from baseline in femoral neck cortical and mean lumbar spine (L1, L2) trabecular BMD

	Placebo (n=4)	Sotatercept 0.3 mg/kg (n=6)	Sotatercept 0.5 mg/kg (n=5)	Sotatercept 0.7 mg/kg (n=6)
Femoral neck cortical				
Baseline BMD, mg/cm^3	640.5	667.0	593.1	594.8
% change from baseline BMD (n)	-1.4 (4)	-1.4 (5)	1.6 (5)	-0.1 (4)
Mean lumbar spine (L1, L2) trabecular				
Baseline BMD, mg/cm^3	140.1	125.6	149.1	118.7
% change from baseline BMD (n)	10.9 (4)	8.0 (6)	0.5 (5)	4.5 (6)

Note: The n reflects the number of randomized subjects with paired QCT assessments; actual number of subjects available for each parameter may vary.

9.8.3 CONCLUSIONS

[00528] Based on the baseline biomarker data, subjects in this example tended to have high-turnover ROD. The placebo group had decreased cortical bone mass, increased trabecular bone mass, and increased vascular calcification that occurred at a rate similar to other large studies in ESKD (Raggi *et al.*, 2011, Nephrol. Dial. Transplant. 26:1327-1339), as expected for high-turnover ROD.

[00529] QCT data measured at baseline and day 225 (which is after up to eight 28-day dose cycles), indicated that treatment of high-turnover ROD with sotatercept resulted in an effect on multiple parameters of CKD-MBD, including slowed progression of vascular calcification, increased femoral neck cortical bone mass, and a slowed increase in lumbar spine bone mass. The decrease in vascular calcification and the increase in bone mass are consistent with the histological findings in the mouse model of *ldlr-/-* high-fat fed, 5/6 nephrectomy for vascular calcification (Fang *et al.*, 2014, Abstract, ASN Kidney Week 2014, November 11-16, 2014, Philadelphia, PA).

9.9 EXAMPLE 9: ACTRIIA-HFC (SEQ ID NO:7, "SOTATERCEPT") AFFECTS MULTIPLE MANIFESTATIONS OF END-STAGE KIDNEY DISEASE

9.9.1 BACKGROUND

[00530] This example presents data from a clinical study. Examples 7 and 8 (Section Section 9.7 and Section 9.8) also present data from this study.

[00531] This ongoing, randomized, single-blind, placebo-controlled study evaluated the pharmacokinetics, safety, and hemoglobin effect of ActRIIA-hFc (SEQ ID NO:7; "sotatercept"), an ActRIIA-IgG1 fusion protein ligand trap for the correction of anemia in hemodialysis subjects, and explored its effects on vascular calcification and bone mineral density using quantitative computed tomography. This example provides interim results for the 0.3, 0.5, and 0.7 mg/kg dose groups.

9.9.2 METHODS

[00532] Erythropoietin-stimulating agent (ESA)-responsive subjects were washed out of ESA effects until hemoglobin was <10 g/dL and randomized to placebo or sotatercept administered subcutaneously every 28 days for ≤8 dose cycles. Treatment failures (hemoglobin<9 g/dL) were rescued with ESA or transfusion; intrasubject dose escalation was not permitted. Quantitative computed tomography scans of hip, lumbar spine, and abdominal aorta were obtained at baseline and after the 225-day treatment phase. Reported are interim results for pharmacokinetics, safety, home blood pressure, hemoglobin, vascular calcification, and bone mineral density effects.

9.9.3 RESULTS

[00533] Among subjects treated with placebo (n=9) or sotatercept 0.3 mg/kg (n=9), 0.5 mg/kg (n=8), and 0.7 mg/kg (n=9), adverse events were mostly mild/moderate, unrelated to study drug, relatively similar in type/severity between groups, and generally consistent with subject medical histories. Two deaths occurred in the placebo group. There were no dose-dependent changes in home blood pressure. In the 225-day treatment phase, hemoglobin was >10 g/dL in 33%, 33%, 63%, and 78% of subjects treated with placebo or sotatercept 0.3, 0.5, or 0.7 mg/kg, respectively. Paired quantitative computed tomographies obtained in 4, 6, 5, and 6 subjects treated with placebo or sotatercept 0.3, 0.5, or 0.7 mg/kg, respectively, showed <15%

progression of vascular calcification in 33%, 80%, 80%, and 100%, and >2% increase in femoral neck cortical bone mineral density in 0%, 20%, 40%, and 75%, respectively.

9.9.4 CONCLUSIONS

[00534] Sotatercept was tolerated with an acceptable safety profile in hemodialysis, without increases in home blood pressure. There were dose-dependent responses to sotatercept in hemoglobin, vascular calcification, and bone mineral density.

9.10 EXAMPLE 10: DISORDERD ACTRIIA SIGNALING IN CKD CONTRIBUTES TO ATHEROSCLEROTIC CALCIFICATION AND RENAL FIBROSIS

[00535] This example relates to the experiments described and performed in other examples described herein, including, Example 4.

9.10.1 METHODS

9.10.1.1 Production of animal models

[00536] The atherosclerotic *low density lipoprotein receptor* deficient *(ldlr-/-)* males (C57Bl/6J background) were purchased from Jackson Laboratories, and fed high fat diet (42% calories from fat) beginning at 12 weeks of age. The mice are obese, insulin resistant at 22 weeks of age, diabetic at 28 weeks of age and hypercholesterolemic.

[00537] A two-step procedure was utilized to create chronic kidney disease as described previously. (Davies MR, *et al.* J Am Soc Nephrol 2003; 14: 1559-1567; Davies MR, *et al.* J Am Soc Nephrol 2005; 16: 917-928). Electrocautery was applied to the right kidney through a 2 cm flank incision at 12 weeks post-natal, followed by left total nephrectomy at 14 weeks of age. The intensity of the cautery was varied to produce moderate (CKD-3) renal injury that was confirmed by inulin clearances at age 20 weeks (Fig. 33A, B). Control animals received sham operations in which the appropriate kidney was exposed and mobilized but not treated in any other way. Five groups of mice were used in this study (Fig. 23B). The first group was wild type C57Bl/6J mice fed a regular chow diet (WT). This was the normal renal function and diet group used for normative control values. The second group was *ldlr-/-* mice that were fed a high fat diet and sham operated (Sham). This group served as the control group to determine the effect of kidney disease. The third group was *ldlr-/-* mice with GFR reduced equivalent to human CKD stage 3 fed high fat diet (CKD-3) with euthanasia at 22 weeks, the baseline vascular

calcification group (CKD-3) used in Fig. 25A, B, C. The fourth group was *ldlr-/-* mice with CKD-3 receiving subcutaneous injections of vehicle (phosphate buffered saline) twice a week beginning at 22 weeks until euthanasia at 28 weeks (CKD-3 V). The fifth group was *ldlr-/-* mice with CKD-3 receiving subcutaneous injections of mActRIIA-Fc (Celgene, Summit, NJ), 10 mg/kg twice a week beginning at 22 weeks until euthanasia at 28 weeks (CKD-3 R). The dose used was previously shown in PK/PD studies to be an efficacious dose for stimulation of bone formation.

[00538] The second model of CKD used was the murine homolog of X-linked Alport's syndrome, which is a deficiency in the gene for the α5 chain of type IV collagen, COL4A5. (Rheault MN, *et al.* J. the Am. Soc. Neph. 2004; 15: 1466-1474). This is a model of spontaneous kidney disease, and was used throughout the results to confirm the effects of renal ablation induced CKD. Breeding pairs were purchased from Jackson Laboratories and were bred for experiments. Hemizygote males spontaneously developed kidney disease comparable with human CKD stage 3-4 at 150 days after birth and developed hematuria by day 75 of life.

[00539] In all cases, euthanasia was performed under anesthesia. Intraperitoneal anesthesia (xylazine 13 mg/kg and ketamine 87 mg/kg) was used for all procedures. Saphenous vein blood samples were taken 1 week following the second surgery to assess baseline post-surgical renal function. At the time of sacrifice, blood was taken by intracardiac stab, and the heart and aorta dissected en bloc.

9.10.1.2 Inulin clearances

[00540] Inulin clearances were performed at 20 wks or 26 wks if euthanasia was at 28 wks, according to manufacturer instructions (BioPal Inc., Worcester, MA).

9.10.1.3 Chemical Calcification Quantitation

[00541] Aortas were dissected at sacrifice, and all extraneous tissue removed by blunt dissection under a dissecting microscope. Tissues were desiccated for 20-24 hours at 60° C, weighed and crushed to a powder with a pestle and mortar. Calcium was eluted in 1N HCL for 24 hours at 4°C. Calcium content of eluate was assayed using a cresolphthalein complexone method (Sigma, St Louis, MO), according to manufacturer's instructions, and results were corrected for dry tissue weight.

9.10.1.4 Blood tests

[00542] Serum was analyzed on the day of blood draw for blood urea nitrogen (BUN), calcium, and phosphate by standard autoanalyzer laboratory methods performed by an animal

facility. Plasma activin (Fitzgerald Industries, Acton, MA), follistatin (MYBIOSOURCE Inc., San Diego, CA) and follistatin-like 3 (MYBIOSOURCE Inc.) levels were determined in ELISA assays. Serum Dkk1 levels were analyzed with an ELISA (R&D Systems, Minn., MN). For the Elisa assays blood was drawn by saphenous vein or cardiac puncture at the time of euthanasia. All blood samples were placed on ice at collection. Platelet poor EDTA plasma samples were made by a 2-step centrifugation at 6000 rpm for 5 minutes and 14000 rpm for 2 minutes both at 4°C. Samples were stored frozen at – 20°C or below until being used.

9.10.1.5 Histology and immunohistochemistry

[00543] Aortic and kidney tissues were fixed in 10% neutral buffered formalin overnight, then transferred to 70% ethanol at 4 °C, embedded in paraffin and 5 micron sections were prepared. Slides were deparaffinized in xylene and dehydrated in a graded ethanol series and then rehydrated. Mason Trichome staining was used to detect kidney and heart fibrosis and Alizarin red staining was used to detect calcification, according to a standard protocols. (Gregory CA, *et al.* Analytical Biochem. 2004; 329: 77-84). For immunostaining, slides were blocked in 20% normal goat serum for 20 min and incubated with primary antibody at 4 °C overnight. Slides were washed in PBS and blocked in 20% normal goat serum for 20 min before incubation with goat-anti rabbit Alexa 488 secondary antibody 1:400 (Life Technologies, A11008) at room temperature for 30 min, or using TSA kit for signal amplification according manufacture instructions (Life Technologies, T 20932). For double immunofluorescent staining sections were blocked in 20% goat serum and sequentially stained with first primary and secondary antibodies and second primary and secondary antibodies using goat-anti rabbit Alexa 488 and Alexa 568 secondary antibodies 1:400 (Life Technologies, A11008 and A11011) or TSA kit for signal amplification according manufacture instructions (Life Technologies, T20932 and T20934). When primary antibodies from same species were used for double staining slides were heated 5 min in citrate buffer in microwave before second staining. (Tóth ZE, J. HistoChem. & CytoChem. 2007; 55: 545-554). Primary antibodies used in this study for immunostaining: rabbit polyclonal anti-ActRIIA antibody 1:250 (Abcam, ab 135634), rabbit polyclonal anti-Inhibin beta A antibody 1:100 (Santa Cruz, sc-50288), rabbit polyclonal anti-CD31 antibody 1:50 (Abcam, ab28364) and rabbit polyclonal anti-beta-catenin antibodies 1:500 (Abcam, ab32572).

9.10.1.6 RT-PCR

[00544] RNA was extracted from aortas and kidneys using RNeasy Mini Kits (Qiagen, Valencia, CA). 1 µg of total RNA was DNase treated and reverse transcribed using iScript cDNA synthesis kit from Bio-Rad (Hercules, Ca) according to manufacturer's instructions on Veriti Termal Cycler (Applied Biosystems). Primers were designed using Vector NTI (Invitrogen, Grand Island, NY) or Primer Express (Life Technologies, Grand Island, NY) software. Following reverse transcription real time was performed using the StepOne Plus real time PCR instrument (Applied Biosystems), SYBR Green from Sigma (St. Louis) and the PCR kit from Invitrogen. Each reaction was performed in triplicate at 95°C, 45 sec, and 60°C, 30 sec, and 60 sec at 72°C for 40 cycles. This was followed by a melt cycle, which consisted of stepwise increase in temperature from 60°C to 95°C. A single predominant peak was observed in the dissociation curve of each gene, supporting the specificity of the PCR product. Ct numbers (threshold values) were set within the exponential phase of PCR and were used to calculate the expression levels of the genes of interest. B2m was used as an internal standard and normalize the values. A standard curve consisting of the c_T versus log cDNA dilutions (corresponding to the log copy numbers) was generated by amplifying serial dilutions of cDNA corresponding to an unknown amount of amplicon. Negative controls were performed by inactivating the reverse transcriptase by boiling for 5 min prior to RT-PCR to insure that genomic DNA was not amplified.

9.10.1.7 Immunoblotting (Western analyses)

[00545] Whole-cell lysate protein were prepared from kidney and aorta of the mice by RIPA Lysis Buffer (Thermo Scientific) containing a protease inhibitor cocktail (Santa Cruz). Lysates (20 µg) were loaded in 8-12% SDS-PAGE gels and immunoblotted with antibodies to inhibin β-A (Santa Cruz), α-tubulin (Santa Cruz), actin-α smooth muscle (Sigma), Runx2 (Cell Signaling), Myocd (Santa Cruz), ACVRL1 (Origene), ACVR1 (Cell Signaling), Erk1/2 (Cell Signaling), phospho- Erk1/2 (Cell Signaling), ACVR1B (Origene), Col1a1 (Santa Cruz), Smad2/3 (Cell Signaling), or phospho-Smad2/3 (Cell Signaling). Immunoprecipitation (IP) assays used the same whole-cell lysate protein. To reduce nonspecific binding, the samples were pre-cleaned using pre- washed protein A agarose beads (Cell Signaling). Pre-cleaned samples were incubated overnight with the phospho-serine antibody (Abcam). IP-antibody complexes were then captured on protein A agarose beads and proteins were detected by immunoblotting analysis.

[00546] Statistical analysis was performed using ANOVA. All data are expressed as mean ± SD, unless other specified in the figure legend. Differences between groups were assessed post hoc using Fisher LSD method and considered significant at $p < 0.05$. Data for all groups represent an "n" of 7-15. For real time PCR analysis, a minimum of 3 samples were used in each experimental group. For the vascular calcification data in Fig. 26C, the boxes represent median and interquartile ranges (from 25th to 75th percentile), and error bars present 1.5-fold of the interquartile range below 25th and above 75th percentile. Medians were compared using ANOVA Holm–Sidak method for multiple comparisons, $p<0.05$, as critical level for significant difference.

9.10.2 RESULTS

9.10.2.1 Experimental Design, and Kidney function in a model of CKD

[00547] The high fat fed *ldlr-/-* mouse is a model of atherosclerotic vascular calcification requiring both the diet and the genotype to produce atherosclerotic vascular calcification. (Davies MR, *et al.* J Am Soc Nephrol 2003; 14: 1559-1567; Towler DA, *et al.* J. Biological Chem. 1998; 273: 30427-30434). Kidney function was reduced analogous to human stage 3 CKD (CKD-3) in *ldlr-/-* high fat fed mice by renal cortical injury and contralateral nephrectomy (Fig. 33A, B). The CKD-3 mice were hyperphosphatemic (**Table 20**) consistent with the onset of hyperphosphatemia in human stage 3b-4 CKD. (Isakova T, *et al.* Kidney Int 2011; 79: 1370-1378).

[00548] **Table 20:** Serum Biochemical Parameters in the Animals Tested

Parameter	**Group 1 Wild Type**	**Group 2 Sham**	**Group 3 CKD-3 V**	**Group 4 CKD-3 R**
Mouse Strain	C57/BJ6	Idlr-/-	Idlr-/-	Idlr-/-
Diet	Chow	High fat	High fat	High fat
Surgery	None	Sham	CKD	CKD
Weeks postnatal	28	28	28	28
Treatment	None	None	Vehicle	mActRIIA-Fc
N	12	15	14	15
BUN (mg/dl)	24.0 ± 4.6	20.6 ± 3.7	37.7 ± 7.6*	36.5 ± 5.8*
Ca (mg/di)	8.3 ± 1.8	8.9 ± 0.9	9.4 ± 0.8	8.8 ± 0.3
Phosphorus (mg/di)	8.9 ± 0.2	7.9 ± 2.3	11.0 ± 1.6*	11.8 ± 1.2*

*:<0.05, groups 3 and 4 compared to group 2.

9.10.2.2 Activin Receptor Type IIA (ActRIIA) levels in CKD

[00549] Aortas from the high fat fed *ldlr-/-* mice with CKD-3 were analyzed for TGFβ superfamily type II receptors which are one ligand binding component of the superfamily receptor heteromultimers composed of type II and type I (ALK) receptors. The Activin type II receptor A (ActRIIA) was expressed in aortic vascular smooth muscle cells (VSMC) (Fig. 24A, B). CKD-3 produced in the *ldlr-/-* high fat fed mice induced ActRIIA down regulation in the aorta (Fig. 24A). Without being bound by any particular theory, this is consistent with internalization and degradation of ActRIIA produced by high circulating ligand levels reported in other tissues. (Simone ND, *et al.* Endocrinology 1998; 139: 1147-1155; Liu ZH, *et al.* J. Endocrinology 2006; 189: 409-421). Endothelial cell ActRIIA was not detected by immunochemical and immunofluorescent detection. (Fig. 24B).

9.10.2.3 Vascular effects of the ActRIIA ligand trap in CKD

[00550] The effects of CKD-induced suppression of ActRIIA levels were analyzed in aortic homogenates from CKD-3 mice treated with vehicle or the ActRIIA ligand trap (mActRIIA-Fc) (Fig. 25A, B). CKD-3 stimulated osteoblastic transition was assessed by expression of mRNA for Runx2 and alkaline phosphatase (Alpl) in the aortas of *ldlr-/-* high fat fed mice. CKD-3 stimulated their expression and mActRIIA-Fc treatment reversed the effects of CKD (Fig. 25A). Both Runx2 and Alpl expression represent biomarkers of osteoblastic transition in the aorta that were reversed by mActRIIA-Fc treatment. Aortic mRNA of smooth muscle 22α or transgelin (Tagln), a biomarker of differentiated vascular smooth muscle cells, (Li L, Miano, *et al. Circulation Res.* 1996; **78:** 188-195) was decreased by CKD-3 and stimulated by mActRIIA-Fc. CKD-3 also caused decreased aortic myocardin (Myocd) mRNA expression, the vascular smooth muscle cell specific transcription factor, but myocardin was not affected by mActRIIA-Fc treatment. In terms of the effects of CKD-3 and mActRIIA-Fc treatment on aortic protein levels of the respective mRNAs studied in Fig. 25A, CKD increased aortic Runx2 and Alpl levels and mActRIIA-Fc normalized them (Fig. 25B, data for Alpl not shown). CKD decreased the aortic levels of Tagln and alpha smooth muscle actin (αSMA), another biomarker of differentiated vascular smooth muscle cells, and mActRIIA-Fc treatment increased them (Fig. 25B, data for Tagln not shown). Myocardin levels were not changed by CKD or mActRIIA-Fc treatment.

[00551] The *ldlr-/-* high fat fed mouse is a model of atherosclerosis, atherosclerotic calcification and type 2 diabetes, (Al-Aly Z, *et al.* Arteriosclerosis, Thrombosis, and Vascular

Biol. 2007; 27: 2589-2596; Towler DA, *et al.* J. Biological Chem. 1998; 273: 30427-30434). Identified, *inter alia*, CKD-3 caused accumulation of calcium deposits in the aortic atheromas in CKD-3 vehicle treated mice (CKD-3 V) (Fig. 26A) and increased total tissue calcium levels (Fig. 26B). Visible calcium deposits were not present in CKD-3 mice treated with mActRIIA-Fc (CKD-3 mActRIIA-Fc). Moreover, mActRIIA-Fc decreased aortic tissue calcium content to levels like those observed in wild type and sham mice, and levels that were significantly below those present at the time of institution of mActRIIA-Fc treatment in the CKD-3 group (Fig. 26B).

9.10.2.4 ActRIIA signaling in the aorta

[00552] Canonical signal transduction by the TGFβ superfamily involves ligand binding to type II receptors activating their serine/threonine kinase activity and stimulating association and phosphorylation of type I receptors, the Alk kinases (*see* diagrammatic representation in Fig. 34). There are seven Alk kinases utilized by the TGFβ superfamily, and Alk4 (ActRIB) is the type I receptor most often associated with ActRIIA signaling. (Abe Y MT, *et al.* Growth Factors (Chur, Switzerland) 2004; 22: 105-110). ActRIIA levels were not decreased in aortic homogenates isolated from CKD-3 mice as evidenced in Fig. 24, and were not associated with decreased tissue levels of Alk4 and Alk1 (Fig. 27A). Alk5 and Alk2, other type 1 receptors associated with ActRIIA signaling, were not detectable. ActRIIA activity was assessed by measuring the effect of receptor heteromultimerization (*e.g.*, phosphorylation of regulatory Smads). CKD-3 decreased aortic phosphosmad 2/3 levels (activated smad 2/3), and mActRIIA-Fc increased them compared to CKD-3V (Fig. 27A, B). Noncanonical ActRIIA signaling (Fig. 34) was also analyzed: (1) map kinase (phospho-Erk1/2) was decreased by CKD and not further affected by mActRIIA-Fc (Fig. 27A), and (2) vascular smooth muscle levels of p38 and JNK were very low. Also, mActRIIA-Fc p-AKT levels remained unaffected indicating that aortic AKT/ PI3 kinase was not affected by ActRIIA signaling. Aortic ActRIIA signaling (phosphosmad 3) was decreased by CKD and stimulated by the ActRIIA ligand trap associated with suppression of osteoblastic transition in the atherosclerotic aortas by the ActRIIA ligand trap (Fig. 25A, B, C).

[00553] Another non-canonical ActRIIA signaling pathway was examined, the Wnt pathway (Fig. 34). Wnt signaling appeared differentially regulated between aortic endothelium and VSMC. Aortic β-catenin, a major canonical Wnt induced transcription factor, was localized by immunofluorescence to endothelial cells, and was not detectable in VSMC (Fig. 28A, B).

Dkk1 levels were analyzed in the VSMC as a biomarker of Wnt activity. CKD increased VSMC Dkk1 levels and mActRIIA-Fc treatment decreased Dkk1 levels in the CKD-3 mice (Fig. 28D). The decrease in aortic Dkk1 levels by mActRIIA-Fc indicates that vascular smooth muscle Wnt signaling was inhibited by the ActRIIA ligand trap. In addition, the effects of the ActRIIA ligand trap to decrease renal Wnt activity as shown below lead to a major decrease in circulating Dkk1 levels (Fig. 28E). The decrease in systemic Dkk1 was most likely to affect the endothelium. This resulted in increased Wnt signaling in the endothelium where beta-catenin is expressed as shown by the increase in aortic Axin2 levels (Fig. 28C). Axin2 is an immediate early gene stimulated by beta-catenin and often used for the assessment of Wnt signaling. (Mao J, Wang J, Liu B, *et al.* Mol. Cell 2001; 7: 801-809).

9.10.2.5 Effects of the ActRIIA ligand trap on αklotho and renal ActRIIA signaling

[00554] Renal αklotho levels and the effects of CKD-3 and mActRIIA-Fc treatment were examined. As shown in Fig. 29A, a significant increase in renal αklotho levels induced by mActRIIA-Fc treatment. Renal ActRIIA levels and ActRIIA signaling were examined. Renal ActRIIA levels were not affected by CKD-3 (Fig. 29B). A primary ActRIIA ligand, activin A, was strongly induced in the CKD-3 mice and suppressed by mActRIIA-Fc treatment (Fig. 31A-D). The lack of available phospho-Alk antibodies impaired detection of type I receptor activation, requiring instead a phosphoserine antibody to immunoprecipitate. Immunoblots of the precipitate were performed with anti-Alk antibodies. Alk4 phosphorylation was not significantly altered in the diseased kidneys of the CKD-3 model or by mActRIIA-Fc treatment (Fig. 29B). Renal phosphosmad 2/3, however, was increased by CKD -3 and decreased by mActRIIA-Fc indicating that the component of renal Smad 2/3 activation in CKD mediated through ActRIIA involved an Alk different from Alk4 (Fig. 29C).

[00555] The effects of the ActRIIA ligand trap were examined on renal fibrosis. Fig. 30A-D shows decreased renal fibrosis in Trichrome stained kidney cortex sections from CKD-3 mActRIIA-Fc treated mice (Fig. 30C and Fig. 30D) when compared to CKD-3V treated mice (Fig. 30A and Fig. 30B). Fig. 35A-D shows low magnification coronal kidney sections with arrows identifying from where the high power sections in Fig. 30A-D were taken and arrow heads delineating the scar reactions from the electrocautery kidney injury. mActRIIA-Fc decreased the scar reaction consistent with the role of activin in wound healing. Furthermore, mActRIIA-Fc decreased the proteinuria stimulated by CKD-3 (Fig. 30E) consistent with the decrease in renal phosphosmad 2/3 and fibrosis induced by mActRIIA-Fc treatment.

9.10.2.6 Potential ActRIIA ligands in CKD

[00556] There are multiple potential ActRIIA ligands including activins A and B, growth and differentiation factor 11 (GDF11), bone morphogenetic proteins 9 and 10 (BMP9 and 10), and other BMPs, such as BMP7, which have lower affinity for the receptor. But a primary ActRIIA ligand is activin. Using two models of CKD, it was identified that systemic circulating activin A levels were 10-fold elevated in the *ldlr-/-* ablative CKD model and 5-fold elevated in the Col4A5 Alport's syndrome mouse model (Fig. 31A, B). Activin B, GDF11, and BMP9 levels were not affected by CKD in these models. Physiologically, there is believed to be a small amount of free activin in the circulation. Without being bound by any particular theory, this may be due to levels of inhibitors stoichiometrically equaling activin levels. Activins associate with circulating inhibitory factors, follistatin and follistatin like 3 (fstL3), (Welt C, *et al.* Experimental Biol. and Med. 2002; 227: 724-752) and inhibin whose circulating levels (Fig. 36A, B) and tissue levels were not affected or decreased by CKD-3. The stoichiometry of follistatin, fstL3, and inhibin (*e.g.*, the sum of 620 pg/ml, plus 400 pg/ml of unmeasured inhibin (Sharpe RM, *et al. J. Andrology* 1999; **20:** 94-101)) to Activin A levels (>5000 pg/ml) in the circulation suggests that CKD produces significant free activin levels – a pathologic event making activin A an active circulating factor in CKD. This data indicates that kidney diseases produce one or more circulating ActRIIA ligands that could downregulate vascular ActRIIA.

[00557] Kidney tissues were analyzed from *ldlr-/-* atherosclerotic calcification model for activin A (a homodimer of Inhibin betaA (Inhba)) to identify the source of increased circulating activin. Inhba mRNA was increased by CKD in the kidney (Fig. 31C) and activin (inhibin β-A) protein levels were increased (Fig. 31D). Renal activin expression localized in the peritubular myofibroblasts of CKD-3 mice (Fig. 32A, B).

9.10.3 CONCLUSIONS

[00558] This example demonstrates that mActRIIA-Fc increased aortic ActRIIA signaling assessed by the levels of phosphorylated Smad2/3. Furthermore, mActRIIA-Rc treatment reversed CKD-induced vascular smooth muscle dedifferentiation, osteoblastic transition and neointimal plaque calcification. In the diseased kidneys, mActRIIA-Fc inhibited, rather than stimulated, ActRIIA signaling and decreased renal fibrosis and proteinuria. mActRIIA-Fc treatment decreased renal and circulating Dkk1 levels demonstrating that Wnt activation was downstream of ActRIIA. This example demonstrates that disordered ActRIIA signaling in CKD

contributes to the CKD-MBD and renal fibrosis, identifies ActRIIA signaling as a therapeutic target in CKD, and demonstrates that an activin ligand trap (*e.g.*, mActRIIA-Fc) can be utilized in the treatment of CKD.

10. DESCRIPTION OF THE SEQUENCES

[00559] Table 21: Sequence Information

SEQ ID NO:	Description	Sequence
1	human ActRIIA precursor polypeptide	MGAAAKLAFAVFLISCSSGAILGRSETQECLFFNA NWEKDRTNQTGVEPCYGDKDKRRHCFATWKNIS GSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYF CCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPPY YNILLYSLVPLMLIAGIVICAFWVYRHHKMAYPP VLVPTQDPGPPPPSPLLGLKPLQLLEVKARGRFGC VWKAQLLNEYVAVKIFPIQDKQSWQNEYEVYSLP GMKHENILQFIGAEKRGTSVDVDLWLITAFHEKG SLSDFLKANVVSWNELCHIAETMARGLAYLHEDI PGLKDGHKPAISHRDIKSKNVLLKNNLTACIADFG LALKFEAGKSAGDTHGQVGTRRYMAPEVLEGAI NFQRDAFLRIDMYAMGLVLWELASRCTAADGPV DEYMLPFEEEIGQHPSLEDMQEVVVHKKKRPVLR DYWQKHAGMAMLCETIEECWDHDAEARLSAGC VGERITQMQRLTNIITTEDIVTVVTMVTNVDFPPK ESSL
2	human ActRIIA soluble (extracellular), processed polypeptide sequence	ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDK DKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR TDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEV TQPTSNPVTPKPP
3	human ActRIIA soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted	ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDK DKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR TDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEM
4	nucleic acid sequence encoding human ActRIIA precursor protein	ATGGGAGCTGCTGCAAAGTTGGCGTTTGCCGTC TTTCTTATCTCCTGTTCTTCAGGTGCTATACTTG GTAGATCAGAAACTCAGGAGTGTCTTTTCTTTA ATGCTAATTGGGAAAAAGACAGAACCAATCAA ACTGGTGTTGAACCGTGTTATGGTGACAAAGAT AAACGGCGGCATTGTTTTGCTACCTGGAAGAAT ATTTCTGGTTCCATTGAAATAGTGAAACAAGGT TGTTGGCTGGATGATATCAACTGCTATGACAGG ACTGATTGTGTAGAAAAAAAAGACAGCCCTGA AGTATATTTTTGTTGCTGTGAGGGCAATATGTG TAATGAAAAGTTTTCTTATTTTCCAGAGATGGA

SEQ ID NO:	Description	Sequence
		AGTCACACAGCCCACTTCAAATCCAGTTACACC TAAGCCACCCTATTACAACATCCTGCTCTATTCC TTGGTGCCACTTATGTTAATTGCGGGGATTGTC ATTTGTGCATTTTGGGTGTACAGGCATCACAAG ATGGCCTACCCTCCTGTACTTGTTCCAACTCAA GACCCAGGACCACCCCCACCTTCTCCATTACTA GGGTTGAAACCACTGCAGTTATTAGAAGTGAAA GCAAGGGGAAGATTTGGTTGTGTCTGGAAAGCC CAGTTGCTTAACGAATATGTGGCTGTCAAAATA TTTCCAATACAGGACAAACAGTCATGGCAAAAT GAATACGAAGTCTACAGTTTGCCTGGAATGAAG CATGAGAACATATTACAGTTCATTGGTGCAGAA AAACGAGGCACCAGTGTTGATGTGGATCTTTGG CTGATCACAGCATTTCATGAAAAGGGTTCACTA TCAGACTTTCTTAAGGCTAATGTGGTCTCTTGG AATGAACTGTGTCATATTGCAGAAACCATGGCT AGAGGATTGGCATATTTACATGAGGATATACCT GGCCTAAAAGATGGCCACAAACCTGCCATATCT CACAGGGACATCAAAAGTAAAAATGTGCTGTT GAAAAACAACCTGACAGCTTGCATTGCTGACTT TGGGTTGGCCTTAAAATTTGAGGCTGGCAAGTC TGCAGGCGATACCCATGGACAGGTTGGTACCCG GAGGTACATGGCTCCAGAGGTATTAGAGGGTG CTATAAACTTCGAAAGGGATGCATTTTTGAGGA TAGATATGTATGCCATGGGATTAGTCCTATGGG AACTGGCTTCTCGCTGTACTGCTGCAGATGGAC CTGTAGATGAATACATGTTGCCATTTGAGGAGG AAATTGGCCAGCATCCATCTCTTGAAGACATGC AGGAAGTTGTTGTGCATAAAAAAAAGAGGCCT GTTTTAAGAGATTATTGGCAGAAACATGCTGGA ATGGCAATGCTCTGTGAAACCATTGAAGAATGT TGGGATCACGACGCAGAAGCCAGGTTATCAGCT GGATGTGTAGGTGAAAGAATTACCCAGATGCA GAGACTAACAAATATTATTACCACAGAGGACAT TGTAACAGTGGTCACAATGGTGACAAATGTTGA CTTTCCTCCCAAAGAATCTAGTCTATGA
5	nucleic acid sequence encoding a human ActRIIA soluble (extracellular) polypeptide	ATACTTGGTAGATCAGAAACTCAGGAGTGTCTT TTCTTTAATGCTAATTGGGAAAAAGACAGAACC AATCAAACTGGTGTTGAACCGTGTTATGGTGAC AAAGATAAACGGCGGCATTGTTTTGCTACCTGG AAGAATATTTCTGGTTCCATTGAAATAGTGAAA CAAGGTTGTTGGCTGGATGATATCAACTGCTAT GACAGGACTGATTGTGTAGAAAAAAAAGACAG CCCTGAAGTATATTTTTGTTGCTGTGAGGGCAA

SEQ ID NO:	Description	Sequence
		TATGTGTAATGAAAAGTTTTCTTATTTTCCAGAG ATGGAAGTCACACAGCCCACTTCAAATCCAGTT ACACCTAAGCCACCC
6	fusion protein comprising a soluble extracellular domain of ActRIIA fused to an Fc domain	THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDX1VSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK X2VSNKALPVPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGPFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNX3HYTQKSLSLSPGK* (wherein X1 is D or A; X2 is K or A and X3 is N or A)
7	Extracellular domain of human ActRIIA fused to a human Fc domain	ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDK DKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR TDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEV TQPTSNPVTPKPPTGGGTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
8	Leader sequence of Honey bee mellitin (HBML)	MKFLVNVALVFMVVYISYIYA
9	Leader sequence of Tissue Plasminogen Activator (TPA)	MDAMKRGLCCVLLLCGAVFVSP
10	Native ActRIIA leader	MGAAAKLAFAVFLISCSSGA
11	ActRIIA-hFc and mActRIIA-Fc N-terminal sequence	ILGRSETQE
12	ActRIIA-Fc Protein with deletion of the C-terminal 15 amino acids of the extracellular domain of ActRIIA	ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDK DKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR TDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMTG GGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPVPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:	Description	Sequence
13	Unprocessed ActRIIA-hFc with TPA leader sequence	MDAMKRGLCCVLLLCGAVFVSPGAAILGRSETQE CLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFA TWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKD SPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPV TPKPPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVM HEALHNHYTQKSLSLSPGK
14	Nucleic acid sequence encoding Unprocessed ActRIIA-hFc with TPA leader sequence	ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTG CTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCC GGCGCCGCTATACTTGGTAGATCAGAAACTCAG GAGTGTCTTTTTTTAATGCTAATTGGGAAAAAG ACAGAACCAATCAAACTGGTGTTGAACCGTGTT ATGGTGACAAAGATAAACGGCGGCATTGTTTTG CTACCTGGAAGAATATTTCTGGTTCCATTGAAT AGTGAAACAAGGTTGTTGGCTGGATGATATCAA CTGCTATGACAGGACTGATTGTGTAGAAAAAAA AGACAGCCCTGAAGTATATTTCTGTTGCTGTGA GGGCAATATGTGTAATGAAAAGTTTTCTTATTT TCCGGAGATGGAAGTCACACAGCCCACTTCAAA TCCAGTTACACCTAAGCCACCCACCGGTGGTGG AACTCACACATGCCCACCGTGCCCAGCACCTGA ACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCC CCCAAAACCCAAGGACACCCTCATGATCTCCCG GACCCCTGAGGTCACATGCGTGGTGGTGGACGT GAGCCACGAAGACCCTGAGGTCAAGTTCAACT GGTACGTGGACGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTACAACAG CACGTACCGTGTGGTCAGCGTCCTCACCGTCCT GCACCAGGACTGGCTGAATGGCAAGGAGTACA AGTGCAAGGTCTCCAACAAAGCCCTCCCAGTCC CCATCGAGAAAACCATCTCCAAAGCCAAAGGG CAGCCCCGAGAACCACAGGTGTACACCCTGCCC CCATCCCGGGAGGAGATGACCAAGAACCAGGT CAGCCTGACCTGCCTGGTCAAAGGCTTCTATCC CAGCGACATCGCCGTGGAGTGGGAGAGCAATG GGCAGCCGGAGAACAACTACAAGACCACGCCT CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCT ATAGCAAGCTCACCGTGGACAAGAGCAGGTGG CAGCAGGGGAACGTCTTCTCATGCTCCGTGATG CATGAGGCTCTGCACAACCACTACACGCAGAA

SEQ ID NO:	Description	Sequence
		GAGCCTCTCCCTGTCTCCGGTAAATGAGAATTC
15	human ActRIIB soluble (extracellular), processed polypeptide sequence with the N-terminal 6 amino acids of the EC domain deleted and the C-terminal 4 amino acids of the EC domain deleted (amino acids 25-130 of SEQ ID NO:28) and with an L79D mutation	ETRECIYYNANWELERTNQSGLERCEGEQDKRLH CYASWRNSSGTIELVKKGCWDDDFNCYDRQECV ATEENPQVYFCCCEGNFCNERFTHLPEAGGP EVTYEPPP
16	human ActRIIB precursor protein sequence (A64)	MTAPWVALALLWGSLWPGSGRGEAETRECIYYN ANWELERTNQSGLERCEGEQDKRLHCYASWANS SGTIELVKKGCWLDDFNCYDRQECVATEENPQV YFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT LLTVLAYSLLPIGGLSLIVLLAFWMYRHRKPPYGH VDIHEDPGPPPPSPLVGLKPLQLLEIKARGRFGCV WKAQLMNDFVAVKIFPLQDKQSWQSEREIFSTPG MKHENLLQFIAAEKRGSNLEVELWLITAFHDKGS LTDYLKGNIITWNELCHVAETMSRGLSYLHEDVP WCRGEGHKPSIAHRDFKSKNVLLKSDLTAVLADF GLAVRFEPGKPPGDTHGQVGTRRYMAPEVLEGAI NFQRDAFLRIDMYAMGLVLWELVSRCKAADGPV DEYMLPFEEEIGQHPSLEELQEVVVHKKMRPTIKD HWLKHPGLAQLCVTIEECWDHDAEARLSAGCVE ERVSLIRRSVNGTTSDCLVSLVTSVTNVDLPPKES SI
17	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 19-134 of SEQ ID NO:16)	SGRGEAETRECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWANSSGTIELVKKGCWLDDFNCY DRQECVATEENPQVYFCCCEGNFCNERFTHLPEA GGPEVTYEPPPTAPT
18	human ActRIIB soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted (amino acids 19-119 of SEQ ID NO:16)	SGRGEAETRECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWANSSGTIELVKKGCWLDDFNCY DRQECVATEENPQVYFCCCEGNFCNERFTHLPEA
19	nucleic acid sequence encoding a human ActRIIB (A64) precursor protein	ATGACGGCGCCCTGGGTGGCCCTCGCCCTCCTC TGGGGATCGCTGTGGCCCGGCTCTGGGCGTGGG GAGGCTGAGACACGGGAGTGCATCTACTACAA

SEQ ID NO:	Description	Sequence
		CGCCAACTGGGAGCTGGAGCGCACCAACCAGA GCGGCCTGGAGCGCTGCGAAGGCGAGCAGGAC AAGCGGCTGCACTGCTACGCCTCCTGGGCCAAC AGCTCTGGCACCATCGAGCTCGTGAAGAAGGG CTGCTGGCTAGATGACTTCAACTGCTACGATAG GCAGGAGTGTGTGGCCACTGAGGAGAACCCCC AGGTGTACTTCTGCTGCTGTGAAGGCAACTTCT GCAACGAGCGCTTCACTCATTTGCCAGAGGCTG GGGGCCCGGAAGTCACGTACGAGCCACCCCCG ACAGCCCCCACCCTGCTCACGGTGCTGGCCTAC TCACTGCTGCCCATCGGGGGCCTTTCCCTCATC GTCCTGCTGGCCTTTTGGATGTACCGGCATCGC AAGCCCCCCTACGGTCATGTGGACATCCATGAG GACCCTGGGCCTCCACCACCATCCCCTCTGGTG GGCCTGAAGCCACTGCAGCTGCTGGAGATCAA GGCTCGGGGGCGCTTTGGCTGTGTCTGGAAGGC CCAGCTCATGAATGACTTTGTAGCTGTCAAGAT CTTCCCACTCCAGGACAAGCAGTCGTGGCAGAG TGAACGGGAGATCTTCAGCACACCTGGCATGAA GCACGAGAACCTGCTACAGTTCATTGCTGCCGA GAAGCGAGGCTCCAACCTCGAAGTAGAGCTGT GGCTCATCACGGCCTTCCATGACAAGGGCTCCC TCACGGATTACCTCAAGGGGAACATCATCACAT GGAACGAACTGTGTCATGTAGCAGAGACGATG TCACGAGGCCTCTCATACCTGCATGAGGATGTG CCCTGGTGCCGTGGCGAGGGCCACAAGCCGTCT ATTGCCCACAGGGACTTTAAAAGTAAGAATGTA TTGCTGAAGAGCGACCTCACAGCCGTGCTGGCT GACTTTGGCTTGGCTGTTCGATTTGAGCCAGGG AAACCTCCAGGGGACACCCACGGACAGGTAGG CACGAGACGGTACATGGCTCCTGAGGTGCTCGA GGGAGCCATCAACTTCCAGAGAGATGCCTTCCT GCGCATTGACATGTATGCCATGGGGTTGGTGCT GTGGGAGCTTGTGTCTCGCTGCAAGGCTGCAGA CGGACCCGTGGATGAGTACATGCTGCCCTTTGA GGAAGAGATTGGCCAGCACCCTTCGTTGGAGG AGCTGCAGGAGGTGGTGGTGCACAAGAAGATG AGGCCCACCATTAAAGATCACTGGTTGAAACAC CCGGGCCTGGCCCAGCTTTGTGTGACCATCGAG GAGTGCTGGGACCATGATGCAGAGGCTCGCTTG TCCGCGGGCTGTGTGGAGGAGCGGGTGTCCCTG ATTCGGAGGTCGGTCAACGGCACTACCTCGGAC TGTCTCGTTTCCCTGGTGACCTCTGTCACCAATG TGGACCTGCCCCCTAAAGAGTCAAGCATCTAA

SEQ ID NO:	Description	Sequence
20	fusion protein comprising a soluble extracellular domain of ActRIIB (A64; SEQ ID NO:17) fused to an Fc domain	SGRGEAETRECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWANSSGTIELVKKGCWLDDFNCY DRQECVATEENPQVYFCCCEGNFCNERFTHLPEA GGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
21	fusion protein comprising a soluble extracellular domain of ActRIIB (A64) with the C-terminal 15 amino acids deleted (SEQ ID NO:18) fused to an Fc domain	SGRGEAETRECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWANSSGTIELVKKGCWLDDFNCY DRQECVATEENPQVYFCCCEGNFCNERFTHLPEA GGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK
22	human ActRIIB soluble (extracellular), processed polypeptide sequence with the N-terminal 6 amino acids of the EC domain deleted and the C-terminal 5 amino acids of the EC domain deleted (amino acids 25-129 of SEQ ID NO:28) and with an L79D mutation	ETRECIYYNANWELERTNQSGLERCEGEQDKRLH CYASWRNSSGTIELVKKGCWDDDFNCYDRQECV ATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVT YEPP
23	human ActRIIB soluble (extracellular), processed polypeptide sequence with the N-terminal 6 amino acids of the EC domain deleted and the C-terminal 3 amino acids of the EC domain deleted (amino acids 25-131 of SEQ ID NO:28) and with an L79D mutation	ETRECIYYNANWELERTNQSGLERCEGEQDKRLH CYASWRNSSGTIELVKKGCWDDDFNCYDRQECV ATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVT YEPPPT

SEQ ID NO:	Description	Sequence
24	Unprocessed ActRIIB-Fc fusion protein with the N-terminal 6 amino acids of the EC domain deleted and the C-terminal 3 amino acids of the EC domain deleted (amino acids 25-131 of SEQ ID NO:28) and with an L79D mutation and with TPA leader sequence	MDAMKRGLCCVLLLCGAVFVSPGAAETRECIYY NANWELERTNQSGLERCEGEQDKRLHCYASWRN SSGTIELVKKGCWDDDFNCYDRQECVATEENPQV YFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTGGG THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK*
25	Processed ActRIIB-Fc fusion protein with the N-terminal 6 amino acids of the EC domain deleted and the C-terminal 3 amino acids of the EC domain deleted (amino acids 25-131 of SEQ ID NO:28) and with an L79D mutation	ETRECIYYNANWELERTNQSGLERCEGEQDKRLH CYASWRNSSGTIELVKKGCWDDDFNCYDRQECV ATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVT YEPPPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
26	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:16)	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWANSSGTIELVKKGCWLDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG GPEVTYEPPPTAPT
27	human ActRIIB soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted (amino acids 20-119 of SEQ ID NO:16)	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWANSSGTIELVKKGCWLDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPE A
28	human ActRIIB precursor protein sequence (R64)	MTAPWVALALLWGSLWPGSGRGEAETRECIYYN ANWELERTNQSGLERCEGEQDKRLHCYASWRNS SGTIELVKKGCWLDDFNCYDRQECVATEENPQV YFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT LLTVLAYSLLPIGGLSLIVLLAFWMYRHRKPPYGH VDIHEDPGPPPPSPLVGLKPLQLLEIKARGRFGCV WKAQLMNDFVAVKIFPLQDKQSWQSEREIFSTPG MKHENLLQFIAAEKRGSNLEVELWLITAFHDKGS LTDYLKGNIITWNELCHVAETMSRGLSYLHEDVP WCRGEGHKPSIAHRDFKSKNVLLKSDLTAVLADF GLAVRFEPGKPPGDTHGQVGTRRYMAPEVLEGAI NFQRDAFLRIDMYAMGLVLWELVSRCKAADGPV

SEQ ID NO:	Description	Sequence
		DEYMLPFEEEIGQHPSLEELQEVVVHKKMRPTIKD HWLKHPGLAQLCVTIEECWDHDAEARLSAGCVE ERVSLIRRSVNGTTSDCLVSLVTSVTNVDLPPKES SI
29	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 19-134 of SEQ ID NO:28)	SGRGEAETRECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY DRQECVATEENPQVYFCCCEGNFCNERFTHLPEA GGPEVTYEPPPTAPT
30	human ActRIIB soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted (amino acids 19-119 of SEQ ID NO:28)	SGRGEAETRECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY DRQECVATEENPQVYFCCCEGNFCNERFTHLPEA
31	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:28)	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG GPEVTYEPPPTAPT
32	human ActRIIB soluble (extracellular), processed polypeptide sequence with the C-terminal 15 amino acids deleted (amino acids 20-119 of SEQ ID NO:28)	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPE A
33	human ActRIIB soluble (extracellular), processed polypeptide sequence with the N-terminal 6 amino acids of the EC domain deleted and the C-terminal 3 amino acids of the EC domain deleted (amino acids 25-131 of SEQ ID NO:16) and with an L79D mutation	ETRECIYYNANWELERTNQSGLERCEGEQDKRLH CYASWANSSGTIELVKKGCWDDDFNCYDRQECV ATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVT YEPPPT
34	Unprocessed ActRIIB-Fc fusion protein with the N-terminal 6 amino acids of the EC domain deleted and the C-terminal 3 amino	MDAMKRGLCCVLLLCGAVFVSPGAAETRECIYY NANWELERTNQSGLERCEGEQDKRLHCYASWAN SSGTIELVKKGCWDDDFNCYDRQECVATEENPQV YFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTGGG THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE

SEQ ID NO:	Description	Sequence
	acids of the EC domain deleted (amino acids 25-131 of SEQ ID NO:16) and with an L79D mutation and with TPA leader sequence	VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK*
35	Processed ActRIIB-Fc fusion protein with the N-terminal 6 amino acids of the EC domain deleted and the C-terminal 3 amino acids of the EC domain deleted (amino acids 25-131 of SEQ ID NO:16) and with an L79D mutation	ETRECIYYNANWELERTNQSGLERCEGEQDKRLH CYASWANSSGTIELVKKGCWDDDFNCYDRQECV ATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVT YEPPPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
36	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:28) with L79D mutation	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWDDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG GPEVTYEPPPTAPT
37	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:16) with L79D mutation	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWANSSGTIELVKKGCWDDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG GPEVTYEPPPTAPT
38	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:28) with L79D mutation fused to an Fc domain with a GGG linker	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWDDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG GPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK*
39	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:16) with L79D	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWANSSGTIELVKKGCWDDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG GPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF

SEQ ID NO:	Description	Sequence
	mutation fused to an Fc domain	NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK*
40	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:28) with L79D mutation fused to an Fc domain and with TPA leader sequence	MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETR ECIYYNANWELERTNQSGLERCEGEQDKRLHCY ASWRNSSGTIELVKKGCWDDDFNCYDRQECVAT EENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEP PPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
41	human ActRIIB soluble (extracellular), processed polypeptide sequence (amino acids 20-134 of SEQ ID NO:16) with L79D mutation fused to an Fc domain and with TPA leader sequence	MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETR ECIYYNANWELERTNQSGLERCEGEQDKRLHCY ASWANSSGTIELVKKGCWDDDFNCYDRQECVAT EENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEP PPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
42	human ActRIIB soluble (extracellular), processed polypeptide sequence having a variant C-terminal sequence (disclosed in WO2007/053775)	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG GPEGPWASTTIPSGGPEATAAAGDQGSGALWLCL EGPAHE
43	human ActRIIB soluble (extracellular), processed polypeptide sequence having a variant C-terminal sequence (disclosed in WO2007/053775) having an L79D mutation	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWDDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG GPEGPWASTTIPSGGPEATAAAGDQGSGALWLCL EGPAHE
44	human ActRIIB soluble (extracellular), processed polypeptide sequence	GRGEAETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWDDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTHLPEAG

SEQ ID NO:	Description	Sequence
	having a variant C-terminal sequence (disclosed in WO2007/053775) having an L79D mutation fused to an Fc domain with a TGGG linker	GPEGPWASTTIPSGGPEATAAAGDQGSGALWLCL EGPAHETGGGTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*
45	Nucleic Acid Sequence Encoding SEQ ID NO:24	ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTG CTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCC GGCGCCGCCGAAACCCGCGAATGTATTTATTAC AATGCTAATTGGGAACTCGAACGGACGAACCA ATCCGGGCTCGAACGGTGTGAGGGGGAACAGG ATAAACGCCTCCATTGCTATGCGTCGTGGAGGA ACTCCTCCGGGACGATTGAACTGGTCAAGAAAG GGTGCTGGGACGACGATTTCAATTGTTATGACC GCCAGGAATGTGTCGCGACCGAAGAGAATCCG CAGGTCTATTTCTGTTGTTGCGAGGGGAATTTCT GTAATGAACGGTTTACCCACCTCCCCGAAGCCG GCGGGCCCGAGGTGACCTATGAACCCCCGCCC ACCGGTGGTGGAACTCACACATGCCCACCGTGC CCAGCACCTGAACTCCTGGGGGGACCGTCAGTC TTCCTCTTCCCCCCAAAACCCAAGGACACCCTC ATGATCTCCCGGACCCCTGAGGTCACATGCGTG GTGGTGGACGTGAGCCACGAAGACCCTGAGGT CAAGTTCAACTGGTACGTGGACGGCGTGGAGGT GCATAATGCCAAGACAAAGCCGCGGGAGGAGC AGTACAACAGCACGTACCGTGTGGTCAGCGTCC TCACCGTCCTGCACCAGGACTGGCTGAATGGCA AGGAGTACAAGTGCAAGGTCTCCAACAAAGCC CTCCCAGCCCCCATCGAGAAAACCATCTCCAAA GCCAAAGGGCAGCCCCGAGAACCACAGGTGTA CACCCTGCCCCCATCCCGGGAGGAGATGACCA AGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG GCTTCTATCCCAGCGACATCGCCGTGGAGTGGG AGAGCAATGGGCAGCCGGAGAACAACTACAAG ACCACGCCTCCCGTGCTGGACTCCGACGGCTCC TTCTTCCTCTATAGCAAGCTCACCGTGGACAAG AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC TCCGTGATGCATGAGGCTCTGCACAACCACTAC ACGCAGAAGAGCCTCTCCCTGTCCCCGGGTAAA TGA
46	fusion protein comprising a soluble extracellular	SGRGEAETRECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY

SEQ ID NO:	Description	Sequence
	domain of ActRIIB (R64; SEQ ID NO:29) fused to an Fc domain	DRQECVATEENPQVYFCCCEGNFCNERFTHLPEA GGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
47	fusion protein comprising a soluble extracellular domain of ActRIIB (R64) with the C-terminal 15 amino acids deleted (SEQ ID NO:30) fused to an Fc domain	SGRGEAETRECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY DRQECVATEENPQVYFCCCEGNFCNERFTHLPEA GGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK
48	Runx2 qRT-PCR primer 1	CCGCACGACAACCGCACCAT
49	Runx2 qRT-PCR primer 2	CGCTCCGGCCCACAAATCTC
50	Alp qRT-PCR primer 1	ACGTGGCTAAGAATGTCATC
51	Alp qRT-PCR primer 2	CTGGTAGGCGATGTCCTTA
52	osterix qRT-PCR primer 1	TAGTGGTTTGGGGTTTGTTTACCGC
53	osterix qRT-PCR primer 2	AACCAAATAACTCTTATTCCCTAAGT
54	Klotho qRT-PCR primer 1	GCTCTCAAAGCCCACATACTG
55	Klotho qRT-PCR primer 1	GCAGCATAACGATAGAGGCC
56	Sm22-alpha qRT-PCR primer 1	GTTCCAGACTGTTGACCTCTTT
57	Sm22-alpha qRT-PCR primer 2	CTGCGCTTTCTTCATAAACC
58	Human Runx2 mRNA	GTGTGAATGCTTCATTCGCCTCACAAACAACCA CAGAACCACAAGTGCGGTGCAAACTTTCTCCAG GAGGACAGCAAGAAGTCTCTGGTTTTTAAATGG TTAATCTCCGCAGGTCACTACCAGCCACCGAGA CCAACAGAGTCATTTAAGGCTGCAAGCAGTATT TACAACAGAGGGTACAAGTTCTATCTGAAAAA AAAAGGAGGGACTATGGCATCAAACAGCCTCT TCAGCACAGTGACACCATGTCAGCAAAACTTCT TTTGGGATCCGAGCACCAGCCGGCGCTTCAGCC CCCCCTCCAGCAGCCTGCAGCCCGGCAAAATGA GCGACGTGAGCCCGGTGGTGGCTGCGCAACAG

SEQ ID NO:	Description	Sequence
		CAGCAGCAACAGCAGCAGCAGCAACAGCAGCA GCAGCAGCAGCAACAGCAGCAGCAGCAGCAGG AGGCGGCGGCGGCGGCTGCGGCGGCGGCGGCG GCTGCGGCGGCGGCAGCTGCAGTGCCCCGGTTG CGGCCGCCCCACGACAACCGCACCATGGTGGA GATCATCGCCGACCACCCGGCCGAACTCGTCCG CACCGACAGCCCCAACTTCCTGTGCTCGGTGCT GCCCTCGCACTGGCGCTGCAACAAGACCCTGCC CGTGGCCTTCAAGGTGGTAGCCCTCGGAGAGGT ACCAGATGGGACTGTGGTTACTGTCATGGCGGG TAACGATGAAAATTATTCTGCTGAGCTCCGGAA TGCCTCTGCTGTTATGAAAAACCAAGTAGCAAG GTTCAACGATCTGAGATTTGTGGGCCGGAGTGG ACGAGGCAAGAGTTTCACCTTGACCATAACCGT CTTCACAAATCCTCCCCAAGTAGCTACCTATCA CAGAGCAATTAAAGTTACAGTAGATGGACCTCG GGAACCCAGAAGGCACAGACAGAAGCTTGATG ACTCTAAACCTAGTTTGTTCTCTGACCGCCTCAG TGATTTAGGGCGCATTCCTCATCCCAGTATGAG AGTAGGTGTCCCGCCTCAGAACCCACGGCCCTC CCTGAACTCTGCACCAAGTCCTTTTAATCCACA AGGACAGAGTCAGATTACAGACCCCAGGCAGG CACAGTCTTCCCCGCCGTGGTCCTATGACCAGT CTTACCCCTCCTACCTGAGCCAGATGACGTCCC CGTCCATCCACTCTACCACCCCGCTGTCTTCCAC ACGGGGCACTGGGCTTCCTGCCATCACCGATGT GCCTAGGCGCATTTCAGATGATGACACTGCCAC CTCTGACTTCTGCCTCTGGCCTTCCACTCTCAGT AAGAAGAGCCAGGCAGGTGCTTCAGAACTGGG CCCTTTTTCAGACCCCAGGCAGTTCCCAAGCAT TTCATCCCTCACTGAGAGCCGCTTCTCCAACCC ACGAATGCACTATCCAGCCACCTTTACTTACAC CCCGCCAGTCACCTCAGGCATGTCCCTCGGTAT GTCCGCCACCACTCACTACCACACCTACCTGCC ACCACCCTACCCCGGCTCTTCCCAAAGCCAGAG TGGACCCTTCCAGACCAGCAGCACTCCATATCT CTACTATGGCACTTCGTCAGGATCCTATCAGTTT CCCATGGTGCCGGGGGGGAGACCGGTCTCCTTCC AGAATGCTTCCGCCATGCACCACCACCTCGAAT GGCAGCACGCTATTAAATCCAAATTTGCCTAAC CAGAATGATGGTGTTGACGCTGATGGAAGCCAC AGCAGTTCCCCAACTGTTTTGAATTCTAGTGGC AGAATGGATGAATCTGTTTGGCGACCATATTGA AATTCCTCAGCAGTGGCCCAGTGGTATCTGGGG GCCACATCCCACACGTATCAATATATACATATA

SEQ ID NO:	Description	Sequence
		TAGAGAGAGTGCATATATATGTATATCGATTAG CTATCTACAAAGTGCCTATTTTTTAGAAGATTTT TCATTCACTCACTCAGTCATGATCTTGCAGCCAT AAGAGGGTAGATATTGAGAAGCAGAAGGCTCA AGAGAGACAATTGCAATCGAGCTTCAGATTGTT TACTATTTAAGATGTACTTTTACAAAGGAACAA AGAAGGGAAAAGGTATTTTTGTTTTTGTTGTTT GGTCTGTTATCATCAATAACCTGTTCATATGCC AATTCAGAGAGGTGGACTCCAGGTTCAGGAGG GAGAAGAGCAAAGCCGCTTCCTCTCTGTGCTTT GAAACTTCACACCCTCACGGTGGCAGCTGTGTA TGGACCAGTGCCCTCCGCAGACAGCTCACAAAA CCAGTTGAGGTGCACTAAAGGGACATGAGGTA GAATGGATGCTTCCATCACAGTACCATCATTCA GAATAACTCTTCCAATTTCTGCTTTCAGACATGC TGCAGGTCCTCATCTGAACTGTTGGGTTCGTTTT TTTTTTTTTTTTTCCTGCTCCAAGAAAGTGACTT CAAAAATAACTGATCAGGATAGATTATTTTATT TTACTTTTTAACACTCCTTCTCCCCTTTTCCCACT GAACCAAAAAAGAAATCCCATCCCTAAAACCTG CCTTCTCCTTTTATGCAAAACTGAAAATGGCAA TACATTATTATAGCCATAATGGTATAGATAGTG ATTGCGTTTGGCTATGTGTTGTTTTCTTTTTTTTT AAATTATGAATATGTGTAAAATCTGAGGTAACT TGCTAACGTGAATGGTCATATAACTTTAAAGAT ATATTTATAATTATTTAATGACATTTGGACCCTT GAAACATTTCTTAGTGTATTGATATGTTGACTTC GGTCTCTAAAAGTGCTCTTTATTAAATAACAAA TTTCTTCAGTGGTCTAGAGCCATATCTGAAATA TTGCTAAGCAATTTCAGTTCATCCAGGCACAAT GTGATTTTAAAAAATACTTCCATCTCCAAATAT TTTAGATATAGATTGTTTTTGTGATGTATGAAG GAAATGTTATGTTTAGTTCTTTCAGATCTTTGAA TGCCTCTAACACAGCTTTGCCTTCTAAAGCGGT AATTAGGGATTTAAAAAACAACCTTTAGCCCTT TATCAGCATGAAATGCTGGAGTGATGTGGTTTT CTAATTTCTTTGGGGTAATTATGACTCTTGTCAT ATTAAAAAGACAAGCACAAGTAAATCATTGAA CTACAGAAAAATGTTCTGTGGTTTCATAGTTAA GCAAAACTCTAAATCGCCAGGCTTCATAGCAAA GACATAGTCAGCTAAAAGCCGCACATGTGGAT AGAGGGTTCAATTATGAGACACCTAGTACAGG AGAGCAAAATTGCACCAGAGATTCTTAACCAAC CAGCCTTACCAAACAACACAACAGGGGAACCC CAATCTGCCTTACCCAAGGCCCCACTGGCAGCT

SEQ ID NO:	Description	Sequence
		TTCCACAGAATTTGCATTTAGAGGAGCAGAATG ACATCACTGTCCTTTGGGAGTAGGTCCTCTGAA AAGGCAGCAGGTTCCAGCAGGTAGCTGAGCTG AGAGGACATATGGCCCACGGGGACCTACAGAC AGCCTTTGACATTTGTATTTCTTACAATGGAGG GCCAAGGAGGGCAAGGGGCTGTGGAGTTTGGT GTCTACTAGTGTGTATGAATTTGAGCTAGAGTC CTTCTGTGGCATGCACTTTGACCACTCCTGGCA GTCACATGGCAGATTTCCAAGTGCAAATCCTTA ATCCAAACAAGGATCATCTAATGACACCACCAG GCCAATCCCTGCTCTCCTCCCCGAAAAGTCAGG GTCCCTTCATTGGAATCCTCCACCCACCCAAGC AGAATTTAGCAGAGATTTGCCTTCAAACCCTAA CGGCCCCCTTGTTCTCTGGTCCTTCTCAAACCCA CCTTTGTAGGCCACCCAGCATTGCAGGACAGCG TGTGGGGCAGCTGGACCTGTGCTTCCTGCCTGG GAGTCTCCCTTGGAATTCATCCTGACTCCTTCTA ATAAAAATGGATGGGAAAGCAAAACACTTTGC CTTCTAAAGGCCGTATACCAAGTATGCTTAGAT AAATAAGCCACTTTTCTATTACTTAAGTAAGAA GGAAGTAGTAATTGATACTATTTATTGTTTGTGT GTGGTAGCTTGAAGCACACCACTGTCCATTTAT TTGTAAGTGTAAAATATGTGTGTTTGTTTCAGC AGCACTTAAAAAAGCCAGTGTCTGGTTACACAT TTCAATTTTAATTAATTGACATAAAAATGCTAC CGCCAGTGCCAGCTGCATCCTATTTAATTAAAA AGGTACTATATTTGTACATTATTTTTTAATGTTA AAAGGGCTTTTTTAAGTTTACAGTACACATACC GAGTGACTTTAGGGATGCTTTTGTGTTGAAATG TTACTATAGTGGCTGCAGGCAGCAACCCAGAAA CACTTTAGAAGCTTTTTTTCCTTGGGAAAAATTC AAGCACTTCTTCCCTCCACCCTCACTCCAACCA CCCCAATGGGGGTAATTCACATTTCTTAGAACA AATTCTGCCCTTTTTTGGTCTAGGGATTAAAATT TTGTTTTTCTTTCTTTCTTTTTTTTTTTTTTCACT GAACCCTTAATTTGCACTGGGTCATGTGTTTGA TTTGTGATTTCAAGACCAAAGCAAAGTCTTACT ACTACTGTGGAACCATGTACTAGTTCCTGGGAA TTAAAATAGCGTGGTTCTCTTTGTAGCACAAAC ATTGCTGGAATTTGCAGTCTTTTCAATGCAGCC ACATTTTTATCCATTTCAGTTGTCTCACAAATTT TAACCCATATCAGAGTTCCAGAACAGGTACCAC AGCTTTGGTTTTAGATTAGTGGAATAACATTCA GCCCAGAACTGAGAAACTCAACAGATTAACTAT CGTTTGCTCTTTAGACGGTCTCACTGCCTCTCAC

SEQ ID NO:	Description	Sequence
		TTGCCAGAGCCCTTTCAAAATGAGCAGAGAAGT CCACACCATTAGGGACCATCTGTGATAAATTCA GAAGGGAGGAGATGTGTGTACAGCTTTAAGGA TTCCCTCAATTCCGAGGAAAGGGACTGGCCCAG AATCCAGGTTAATACATGGAAACACGAAGCATT AGCAAAAGTAATAATTATACCTATGGTATTTGA AAGAACAATAATAAAAGACACTTCTTCCAAACC TTGAATTTGTTGTTTTTAGAAAACGAATGCATTT AAAAATATTTTCTATGTGAGAATTTTTTAGATGT GTGTTTACTTCATGTTTACAAATAACTGTTTGCT TTTTAATGCAGTACTTTGAAATATATCAGCCAA AACCATAACTTACAATAATTTCTTAGGTATTCT GAATAAAATTCCATTTCTTTTGGATATGCTTTAC CATTCTTAGGTTTCTGTGGAACAAAAATATTTG TAGCATTTTGTGTAAATACAAGCTTTCATTTTTA TTTTTTCCAATTGCTATTGCCCAAGAATTGCTTT CCATGCACATATTGTAAAAATTCCGCTTTGTGC CACAGGTCATGATTGTGGATGAGTTTACTCTTA ACTTCAAAGGGACTATTTGTATTGTATGTTGCA ACTGTAAATTGAATTATTTGGCATTTTTCTCATG ATTGTAATATTAATTTGAAGTTTGAATTTAATTT TCAATAAAATGGCTTTTTTGGTTTTGTTA
59	Human Alp mRNA	CCGGGCCTCACTCGGGCCCCGCGGCCGCCTTTA TAAGGCGGCGGGGGTGGTGGCCCGGGCCGCGT TGCGCTCCCGCCACTCCGCGCCCGCTATCCTGG CTCCGTGCTCCCACGCGCTTGTGCCTGGACGGA CCCTCGCCAGTGCTCTGCGCAGGATTGGAACAT CAGTTAACATCTGACCACTGCCAGCCCACCCCC TCCCACCCACGTCGATTGCATCTCTGGGCTCCA GGGATAAAGCAGGTCTTGGGGTGCACCATGATT TCACCATTCTTAGTACTGGCCATTGGCACCTGC CTTACTAACTCCTTAGTGCCAGAGAAAGAGAAA GACCCCAAGTACTGGCGAGACCAAGCGCAAGA GACACTGAAATATGCCCTGGAGCTTCAGAAGCT CAACACCAACGTGGCTAAGAATGTCATCATGTT CCTGGGAGATGGGATGGGTGTCTCCACAGTGAC GGCTGCCCGCATCCTCAAGGGTCAGCTCCACCA CAACCCTGGGGAGGAGACCAGGCTGGAGATGG ACAAGTTCCCCTTCGTGGCCCTCTCCAAGACGT ACAACACCAATGCCCAGGTCCCTGACAGCGCC GGCACCGCCACCGCCTACCTGTGTGGGGTGAAG GCCAATGAGGGCACCGTGGGGGTAAGCGCAGC CACTGAGCGTTCCCGGTGCAACACCACCCAGGG GAACGAGGTCACCTCCATCCTGCGCTGGGCCAA GGACGCTGGGAAATCTGTGGGCATTGTGACCAC

SEQ ID NO:	Description	Sequence
		CACGAGAGTGAACCATGCCACCCCCAGCGCCG CCTACGCCCACTCGGCTGACCGGGACTGGTACT CAGACAACGAGATGCCCCCTGAGGCCTTGAGCC AGGGCTGTAAGGACATCGCCTACCAGCTCATGC ATAACATCAGGGACATTGACGTGATCATGGGG GGTGGCCGGAAATACATGTACCCCAAGAATAA AACTGATGTGGAGTATGAGAGTGACGAGAAAG CCAGGGGCACGAGGCTGGACGGCCTGGACCTC GTTGACACCTGGAAGAGCTTCAAACCGAGATAC AAGCACTCCCACTTCATCTGGAACCGCACGGAA CTCCTGACCCTTGACCCCCACAATGTGGACTAC CTATTGGGTCTCTTCGAGCCAGGGGACATGCAG TACGAGCTGAACAGGAACAACGTGACGGACCC GTCACTCTCCGAGATGGTGGTGGTGGCCATCCA GATCCTGCGGAAGAACCCCAAAGGCTTCTTCTT GCTGGTGGAAGGAGGCAGAATTGACCACGGGC ACCATGAAGGAAAAGCCAAGCAGGCCCTGCAT GAGGCGGTGGAGATGGACCGGGCCATCGGGCA GGCAGGCAGCTTGACCTCCTCGGAAGACACTCT GACCGTGGTCACTGCGGACCATTCCCACGTCTT CACATTTGGTGGATACACCCCCCGTGGCAACTC TATCTTTGGTCTGGCCCCCATGCTGAGTGACAC AGACAAGAAGCCCTTCACTGCCATCCTGTATGG CAATGGGCCTGGCTACAAGGTGGTGGGCGGTG AACGAGAGAATGTCTCCATGGTGGACTATGCTC ACAACAACTACCAGGCGCAGTCTGCTGTGCCCC TGCGCCACGAGACCCACGGCGGGGAGGACGTG GCCGTCTTCTCCAAGGGCCCCATGGCGCACCTG CTGCACGGCGTCCACGAGCAGAACTACGTCCCC CACGTGATGGCGTATGCAGCCTGCATCGGGGCC AACCTCGGCCACTGTGCTCCTGCCAGCTCGGCA GGCAGCCTTGCTGCAGGCCCCCTGCTGCTCGCG CTGGCCCTCTACCCCCTGAGCGTCCTGTTCTGA GGGCCCAGGGCCCGGGCACCCACAAGCCCGTG ACAGATGCCAACTTCCCACACGGCAGCCCCCCC CTCAAGGGGCAGGGAGGTGGGGGCCTCCTCAG CCTCTGCAACTGCAAGAAAGGGGACCCAAGAA ACCAAAGTCTGCCGCCCACCTCGCTCCCCTCTG GAATCTTCCCCAAGGGCCAAACCCACTTCTGGC CTCCAGCCTTTGCTCCCTCCCCGCTGCCCTTTGG CCAACAGGGTAGATTTCTCTTGGGCAGGCAGAG AGTACAGACTGCAGACATTCTCAAAGCCTCTTA TTTTTCTAGCGAACGTATTTCTCCAGACCCAGA GGCCCTGAAGCCTCCGTGGAACATTCTGGATCT GACCCTCCCAGTCTCATCTCCTGACCCTCCCACT

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		CCCATCTCCTTACCTCTGGAACCCCCCAGGCCC TACAATGCTCATGTCCCTGTCCCCAGGCCCAGC CCTCCTTCAGGGGAGTTGAGGTCTTTCTCCTCA GGACAAGGCCTTGCTCACTCACTCACTCCAAGA CCACCAGGGTCCCAGGAAGCCGGTGCCTGGGT GGCCATCCTACCCAGCGTGGCCCAGGCCGGGA AGAGCCACCTGGCAGGGCTCACACTCCTGGGCT CTGAACACACACGCCAGCTCCTCTCTGAAGCGA CTCTCCTGTTTGGAACGGCAAAAAAAAATTTTT TTTTCTCTTTTTGGTGGTGGTTAAAAGGGAACA CAAAACATTTAAATAAAACTTTCCAAATATTTC CGAGGACAAAAAAAAAAA
60	Human Osterix mRNA	CGGCGGGCGGCAGCAGCCTAGGCAGCAGCAGT AGCAGAAGCAGCAGCCGCCGAGCAGCAGCAAG GACTCTGGAGTCAGAGTAGGACTGTAGGACCG GAGCCTGAGTGGAACAGGAGTGGAGCTGGCCT GGGAGAGAGCGGATCCCTCCCAGCACCCTCAG GCCACCCGTTGCCTGCACTCTCCCTGCCAGACC TCCAGAGAGGAGAGACTCGGGACAGCCAGCCC CAGGTTCCCCCAGCTCTCTCCATCTGCCTGGCTC CTTGGGACCCGTTCCCCAGCCTCAGGATGGCGT CCTCCCTGCTTGAGGAGGAAGTTCACTATGGCT CCAGTCCCCTGGCCATGCTGACGGCAGCGTGCA GCAAATTTGGTGGCTCTAGCCCTCTGCGGGACT CAACAACTCTGGGCAAAGCAGGCACAAAGAAG CCGTACTCTGTGGGCAGTGACCTTTCAGCCTCC AAAACCATGGGGGATGCTTATCCAGCCCCCTTT ACAAGCACTAATGGGCTCCTTTCACCTGCAGGC AGTCCTCCAGCACCCACCTCAGGCTATGCTAAT GATTACCCTCCCTTTTCCCACTCATTCCCTGGGC CCACAGGCACCCAGGACCCTGGGCTACTAGTGC CCAAGGGGCACAGCTCTTCTGACTGTCTGCCCA GTGTCTACACCTCTCTGGACATGACACACCCCT ATGGCTCCTGGTACAAGGCAGGCATCCATGCAG GCATTTCACCAGGCCCAGGCAACACTCCTACTC CATGGTGGGATATGCACCCTGGAGGCAACTGGC TAGGTGGTGGGCAGGGCCAGGGTGATGGGCTG CAAGGGACACTGCCCACAGGTCCAGCTCAGCCT CCACTGAACCCCCAGCTGCCCACCTACCCATCT GACTTTGCTCCCCTTAATCCAGCCCCCTACCCA GCTCCCCACCTCTTGCAACCAGGGCCCCAGCAT GTCTTGCCCCAAGATGTCTATAAACCCAAGGCA GTGGGAAATAGTGGGCAGCTAGAAGGGAGTGG TGGAGCCAAACCCCCACGGGGTGCAAGCACTG GGGGTAGTGGTGGATATGGGGGCAGTGGGGCA

SEQ ID NO:	Description	Sequence
		GGGCGCTCCTCCTGCGACTGCCCTAATTGCCAG GAGCTAGAGCGGCTGGGAGCAGCAGCGGCTGG GCTGCGGAAGAAGCCCATCCACAGCTGCCACAT CCCTGGCTGCGGCAAGGTGTATGGCAAGGCTTC GCACCTGAAGGCCCACTTGCGCTGGCACACAGG CGAGAGGCCCTTCGTCTGCAACTGGCTCTTCTG CGGCAAGAGGTTCACTCGTTCGGATGAGCTGGA GCGTCATGTGCGCACTCACACCCGGGAGAAGA AGTTCACCTGCCTGCTCTGCTCCAAGCGCTTTAC CCGAAGCGACCACCTGAGCAAACACCAGCGCA CCCATGGAGAACCAGGCCCGGGTCCCCCTCCCA GTGGCCCCAAGGAGCTGGGGGAGGGCCGCAGC ACGGGGGAAGAGGAGGCCAGTCAGACGCCCCG ACCTTCTGCCTCGCCAGCAACCCCAGAGAAAGC CCCTGGAGGCAGCCCTGAGCAGAGCAACTTGCT GGAGATCTGAGCCGGGTGGAAGGTCTCCCACCC CAGGGCTGCCCTGACAGTCTCTCTTGGCTCTCT AGACCACTGCTTGCCAATCACTCTCTTTACCCC ATGCATGCCATCCTTCGGGGCTCTCTCCCTCTGT CTCCCTCCTGGCCATTCTGGGCTTGGGTATCTCC TTGCATGCCTCCTCAGCTCACCTTCTCTCTTCAC CATGAGACTGGCTTTCCACAAACTCTCATCTCA GGCCCTCCCCTTGTGCCTGATACCTGCACTCCG GCTTCCTAGACTCTGGCCCTGCCACACCAACAC ACTTTCTATTTGGGCTCCCAACACTATTTCTCCA TCTCACTCCTTGACATGTACCCCTTTCTGCTTCT CAAGCTTATTTCCTGCTGTCCCTCAGCCTCCAGG CTTCAGTCTTCCCAACTTCTTACACCATTGCTTT CCATTCTCCAGAACTCTTTTTTCCTTTTTACAAA CACAATGATAATGATAATTTATTGCCCCCTGGT GGCCTCTTCATCAGGGGTATTGGGGTTAGTGAC CTGGCCAGAGGGTGCCAAGAGGGGGGCAGACC AGTGGGGATCTGATCCCAAAGATGGGGTGACC CCAGGGTCAGGGAGGCTGCCCCCAGGCCTGTAT ATTTAACCCCTATGTACCAGGAGTAATGAATAG TAATAATTCTATTTATGTAAGTTATGATGACGG GTCAGGTAGAGTGAGCTGGGGAGGGAAGTGGA TCCATTTCTGCTAAGGAAATTCTAGTCAAATGC ATCTCTGTATAGACAAAATGTTAGTGGAGAAGA TCTTGTTAATAGAATGTCTATCATCAGAATCTC AGTTGATAGGGTTTCTCTTGTAATGAAGTCTCT ACAAATTGGGTTAGCTACATCTCTGCTAAACAG TTGATGGGGTATCTCTTGATTAGGGGGATCCCT AATATCCCCAGCCCCAGCCAGAAGCTGTGAAAC CTCAAGTCCTATGGAGGGGAGAAGGACTGGAA

SEQ ID NO:	Description	Sequence
		TGTACCCCATCTCCCTTGACTGCAGAGCAGGTT CCTCCACTGCCCCACCCCTTAGACACCATGACC CCATCAGGTTAATCCCCTGTTGCCATGGTTATG GAGAGCTTGCAGCTGCCATCTTAGATGTGCTCT TTGGGGAAGCCCATCTAACAGGAGGACATTGGT TTGGGGGTGCACCTCCTGAAGAATGGGTGGGG AAGGCTTTCTCTAGGATCAGATTCAAATAAGTA TGTATTGAGTGCCTACTCTGTGCAAGGCACTAT GCTAGATCTGGTGCCTAGAAGCCCTGAGAAAG AACTTAAAGAGCTAGGAGGACAGAGGCCCCCA AGCTGATCTGGTGGTGCATCCACGCACCCCCAC CCTGGGACTTTGGATGCTCCCATCTCCACCTCC AGTGACTTTTAAAGCCGCTTCGTGCCTTTCCTGT AACGTTGGATCCTCCTTTTCTGTCCCCTGCTGTC TCAAGGCCCCAAGTTAAAGGGTTAAAGCCGCTG GAGCTTGGGGAGAGAACATTGTGGAATGGAAG GGATCATGCCCTTTGTGGAGTCTTTTTTTTTTAA TTTAATAAATAAAAGTTGGATTTGAAAAAAAAA AAAAAAAAAAAAAAA
61	Human Klotho mRNA	CGCGCAGCATGCCCGCCAGCGCCCCGCCGCGCC GCCCGCGGCCGCCGCCGCCGTCGCTGTCGCTGC TGCTGGTGCTGCTGGGCCTGGGCGGCCGCCGCC TGCGTGCGGAGCCGGGCGACGGCGCGCAGACC TGGGCCCGTGTCTCGCGGCCTCCTGCCCCCGAG GCCGCGGGCCTCTTCCAGGGCACCTTCCCCGAC GGCTTCCTCTGGGCCGTGGGCAGCGCCGCCTAC CAGACCGAGGGCGGCTGGCAGCAGCACGGCAA GGGTGCGTCCATCTGGGACACGTTCACCCACCA CCCCCTGGCACCCCCGGGAGACTCCCGGAACGC CAGTCTGCCGTTGGGCGCCCCGTCGCCGCTGCA GCCCGCCACCGGGGACGTAGCCAGCGACAGCT ACAACAACGTCTTCCGCGACACGGAGGCGCTGC GCGAGCTCGGGGTCACTCACTACCGCTTCTCCA TCTCGTGGGCGCGAGTGCTCCCCAATGGCAGCG CGGGCGTCCCCAACCGCGAGGGGCTGCGCTACT ACCGGCGCCTGCTGGAGCGGCTGCGGGAGCTG GGCGTGCAGCCCGTGGTCACCCTGTACCACTGG GACCTGCCCCAGCGCCTGCAGGACGCCTACGGC GGCTGGGCCAACCGCGCCCTGGCCGACCACTTC AGGGATTACGCGGAGCTCTGCTTCCGCCACTTC GGCGGTCAGGTCAAGTACTGGATCACCATCGAC AACCCCTACGTGGTGGCCTGGCACGGCTACGCC ACCGGGCGCCTGGCCCCCGGCATCCGGGGCAG CCCGCGGCTCGGGTACCTGGTGGCGCACAACCT CCTCCTGGCTCATGCCAAAGTCTGGCATCTCTA

SEQ ID NO:	Description	Sequence
		CAATACTTCTTTCCGTCCCACTCAGGGAGGTCA GGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCACAGCATCAAAG AATGTCAAAAATCTCTGGACTTTGTACTAGGTT GGTTTGCCAAACCCGTATTTATTGATGGTGACT ATCCCGAGAGCATGAAGAATAACCTTTCATCTA TTCTGCCTGATTTTACTGAATCTGAGAAAAAGT TCATCAAAGGAACTGCTGACTTTTTTGCTCTTTG CTTTGGACCCACCTTGAGTTTTCAACTTTTGGAC CCTCACATGAAGTTCCGCCAATTGGAATCTCCC AACCTGAGGCAACTGCTTTCCTGGATTGACCTT GAATTTAACCATCCTCAAATATTTATTGTGGAA AATGGCTGGTTTGTCTCAGGGACCACCAAGAGA GATGATGCCAAATATATGTATTACCTCAAAAAG TTCATCATGGAAACCTTAAAAGCCATCAAGCTG GATGGGGTGGATGTCATCGGGTATACCGCATGG TCCCTCATGGATGGTTTCGAGTGGCACAGAGGT TACAGCATCAGGCGTGGACTCTTCTATGTTGAC TTTCTAAGCCAGGACAAGATGTTGTTGCCAAAG TCTTCAGCCTTGTTCTACCAAAAGCTGATAGAG AAAAATGGCTTCCCTCCTTTACCTGAAAATCAG CCCCTAGAAGGGACATTTCCCTGTGACTTTGCT TGGGGAGTTGTTGACAACTACATTCAAGTAGAT ACCACTCTGTCTCAGTTTACCGACCTGAATGTTT ACCTGTGGGATGTCCACCACAGTAAAAGGCTTA TTAAAGTGGATGGGGTTGTGACCAAGAAGAGG AAATCCTACTGTGTTGACTTTGCTGCCATCCAG CCCCAGATCGCTTTACTCCAGGAAATGCACGTT ACACATTTTCGCTTCTCCCTGGACTGGGCCCTG ATTCTCCCTCTGGGTAACCAGTCCCAGGTGAAC CACACCATCCTGCAGTACTATCGCTGCATGGCC AGCGAGCTTGTCCGTGTCAACATCACCCCAGTG GTGGCCCTGTGGCAGCCTATGGCCCCGAACCAA GGACTGCCGCGCCTCCTGGCCAGGCAGGGCGC CTGGGAGAACCCCTACACTGCCCTGGCCTTTGC AGAGTATGCCCGACTGTGCTTTCAAGAGCTCGG CCATCACGTCAAGCTTTGGATAACGATGAATGA GCCGTATACAAGGAATATGACATACAGTGCTGG CCACAACCTTCTGAAGGCCCATGCCCTGGCTTG GCATGTGTACAATGAAAAGTTTAGGCATGCTCA GAATGGGAAAATATCCATAGCCTTGCAGGCTGA TTGGATAGAACCTGCCTGCCCTTTCTCCCAAAA GGACAAAGAGGTGGCCGAGAGAGTTTTGGAAT TTGACATTGGCTGGCTGGCTGAGCCCATTTTCG GCTCTGGAGATTATCCATGGGTGATGAGGGACT

SEQ ID NO:	Description	Sequence
		GGCTGAACCAAAGAAACAATTTTCTTCTTCCTT ATTTCACTGAAGATGAAAAAAAGCTAATCCAG GGTACCTTTGACTTTTTGGCTTTAAGCCATTATA CCACCATCCTTGTAGACTCAGAAAAAGAAGATC CAATAAAATACAATGATTACCTAGAAGTGCAA GAAATGACCGACATCACGTGGCTCAACTCCCCC AGTCAGGTGGCGGTAGTGCCCTGGGGGTTGCGC AAAGTGCTGAACTGGCTGAAGTTCAAGTACGG AGACCTCCCCATGTACATAATATCCAACGGAAT CGATGACGGGCTGCATGCTGAGGACGACCAGC TGAGGGTGTATTATATGCAGAATTACATAAACG AAGCTCTCAAAGCCCACATACTGGATGGTATCA ATCTTTGCGGATACTTTGCTTATTCGTTTAACGA CCGCACAGCTCCGAGGTTTGGCCTCTATCGTTA TGCTGCAGATCAGTTTGAGCCCAAGGCATCCAT GAAACATTACAGGAAAATTATTGACAGCAATG GTTTCCCGGGCCCAGAAACTCTGGAAAGATTTT GTCCAGAAGAATTCACCGTGTGTACTGAGTGCA GTTTTTTTCACACCCGAAAGTCTTTACTGGCTTT CATAGCTTTTCTATTTTTTGCTTCTATTATTTCTC TCTCCCTTATATTTTACTACTCGAAGAAAGGCA GAAGAAGTTACAAATAGTTCTGAACATTTTTCT ATTCATTCATTTTGAAATAATTATGCAGACACA TCAGCTGTTAACCATTTGCACCTCTAAGTGTTGT GAAACTGTAAATTTCATACATTTGACTTCTAGA AAACATTTTTGTGGCTTATGACAGAGGTTTTGA AATGGGCATAGGTGATCGTAAAATATTGAATAA TGCGAATAGTGCCTGAATTTGTTCTCTTTTTGGG TGATTAAAAAACTGACAGGCACTATAATTTCTG TAACACACTAACAAAAGCATGAAAAATAGGAA CCACACCAATGCAACATTTGTGCAGAAATTTGA ATGACAAGATTAGGAATATTTTCTTCTGCACCC ACTTCTAAATTTAATGTTTTTCTGGAAGTAGTAA TTGCAAGAGTTCGAATAGAAAGTTATGTACCAA GTAACCATTTCTCAGCTGCCATAATAATGCCTA GTGGCTTCCCCTCTGTCAAATCTAGTTTCCTATG GAAAAGAAGATGGCAGATACAGGAGAGACGAC AGAGGGTCCTAGGCTGGAATGTTCCTTTCGAAA GCAATGCTTCTATCAAATACTAGTATTAATTTAT GTATCTGGTTAATGACATACTTGGAGAGCAAAT TATGGAAATGTGTATTTTATATGATTTTTGAGGT CCTGTCTAAACCCTGTGTCCCTGAGGGATCTGT CTCACTGGCATCTTGTTGAGGGCCTTGCACATA GGAAACTTTTGATAAGTATCTGCGGAAAAACAA ACATGAATCCTGTGATATTGGGCTCTTCAGGAA

SEQ ID NO:	Description	Sequence
		GCATAAAGCAATTGTGAAATACAGTATACCGCA GTGGCTCTAGGTGGAGGAAAGGAGGAAAAAGT GCTTATTATGTGCAACATTATGATTAATCTGATT ATACACCATTTTTGAGCAGATCTTGGAATGAAT GACATGACCTTTCCCTAGAGAATAAGGATGAAA TAATCACTCATTCTATGAACAGTGACACTACTT TCTATTCTTTAGCTGTACTGTAATTTCTTTGAGT TGATAGTTTTACAAATTCTTAATAGGTTCAAAA GCAATCTGGTCTGAATAACACTGGATTTGTTTC TGTGATCTCTGAGGTCTATTTTATGTTTTTGCTG CTACTTCTGTGGAAGTAGCTTTGAACTAGTTTTA CTTTGAACTTTCACGCTGAAACATGCTAGTGAT ATCTAGAAAGGGCTAATTAGGTCTCATCCTTTA ATGCCCCTTAAATAAGTCTTGCTGATTTTCAGA CAGGGAAGTCTCTCTATTACACTGGAGCTGTTT TATAGATAAGTCAATATTGTATCAGGCAAGATA AACCAATGTCATAACAGGCATTGCCAACCTCAC TGACACAGGGTCATAGTGTATAATAATATACTG TACTATATAATATATCATCTTTAGAGGTATGATT TTTTCATGAAAGATAAGCTTTTGGTAATATTCAT TTTAAAGTGGACTTATTAAAATTGGATGCTAGA GAATCAAGTTTATTTTATGTATATATTTTTCTGA TTATAAGAGTAATATATGTTCATTGTAAAAATT TTTAAAACACAGAAACTATATGCAAAGAAAAA ATAAAAATTATCTATAATCTCAGAACCCAGAAA TAGCCACTATTAACATTTCCTACGTATTTTATTT TACATAGATCATATTGTATATAGTTAGTATCTTT ATTAATTTTTATTATGAAACTTTCCTTTGTCATT ATTAGTCTTCAAAAGCATGATTTTTAATAGTTGT TGAGTATTCCACCACAGGAATGTATCACAACTT AACCGTTCCCGTTTGTTAGACTAGTTTCTTATTA ATGTTGATGAATGTTGTTTAAAAATAATTTTGTT GCTACATTTACTTTAATTTCCTTGACTGTAAAGA GAAGTAATTTTGCTCCTTGATAAAGTATTATATT AATAATAAATCTGCCTGCAACTTTTTGCCTTCTT TCATAATC
62	Human Sm22-alpha mRNA	TCACCACGGCGGCAGCCCTTTAAACCCCTCACC CAGCCAGCGCCCCATCCTGTCTGTCCGAACCCA GACACAAGTCTTCACTCCTTCCTGCGAGCCCTG AGGAAGCCTTGTGAGTGCATTGGCTGGGGCTTG GAGGGAAGTTGGGCTGGAGCTGGACAGGAGCA GTGGGTGCATTTCAGGCAGGCTCTCCTGAGGTC CCAGGCGCCAGCTCCAGCTCCCTGGCTAGGGAA ACCCACCCTCTCAGTCAGCATGGGGGCCCAAGC TCCAGGCAGGGTGGGCTGGATCACTAGCGTCCT

SEQ ID NO:	Description	Sequence
		GGATCTCTCTCAGACTGGGCAGCCCCGGGCTCA TTGAAATGCCCCGGATGACTTGGCTAGTGCAGA GGAATTGATGGAAACCACCGGGGTGAGAGGGA GGCTCCCCATCTCAGCCAGCCACATCCACAAGG TGTGTGTAAGGGTGCAGGCGCCGGCCGGTTAGG CCAAGGCTCTACTGTCTGTTGCCCCTCCAGGAG AACTTCCAAGGAGCTTTCCCCAGACATGGCCAA CAAGGGTCCTTCCTATGGCATGAGCCGCGAAGT GCAGTCCAAAATCGAGAAGAAGTATGACGAGG AGCTGGAGGAGCGGCTGGTGGAGTGGATCATA GTGCAGTGTGGCCCTGATGTGGGCCGCCCAGAC CGTGGGCGCTTGGGCTTCCAGGTCTGGCTGAAG AATGGCGTGATTCTGAGCAAGCTGGTGAACAGC CTGTACCCTGATGGCTCCAAGCCGGTGAAGGTG CCCGAGAACCCACCCTCCATGGTCTTCAAGCAG ATGGAGCAGGTGGCTCAGTTCCTGAAGGCGGCT GAGGACTATGGGGTCATCAAGACTGACATGTTC CAGACTGTTGACCTCTTTGAAGGCAAAGACATG GCAGCAGTGCAGAGGACCCTGATGGCTTTGGGC AGCTTGGCAGTGACCAAGAATGATGGGCACTA CCGTGGAGATCCCAACTGGTTTATGAAGAAAGC GCAGGAGCATAAGAGGGAATTCACAGAGAGCC AGCTGCAGGAGGGAAAGCATGTCATTGGCCTTC AGATGGGCAGCAACAGAGGGGCCTCCCAGGCC GGCATGACAGGCTACGGACGACCTCGGCAGAT CATCAGTTAGAGCGGAGAGGGCTAGCCCTGAG CCCGGCCCTCCCCCAGCTCCTTGGCTGCAGCCA TCCCGCTTAGCCTGCCTCACCCACACCCGTGTG GTACCTTCAGCCCTGGCCAAGCTTTGAGGCTCT GTCACTGAGCAATGGTAACTGCACCTGGGCAGC TCCTCCCTGTGCCCCCAGCCTCAGCCCAACTTCT TACCCGAAAGCATCACTGCCTTGGCCCCTCCCT CCCGGCTGCCCCCATCACCTCTACTGTCTCCTCC CTGGGCTAAGCAGGGGAGAAGCGGGCTGGGGG TAGCCTGGATGTGGGCCAAGTCCACTGTCCTCC TTGGCGGCAAAAGCCCATTGAAGAAGAACCAG CCCAGCCTGCCCCCTATCTTGTCCTGGAATATTT TTGGGGTTGGAACTCAAAAAAAAAAAAAAAAAA ATCAATCTTTTCTCAAAAAAAAAAAAAAAAAAA
63	Human Runx2 protein	MASNSLFSTVTPCQQNFFWDPSTSRRFSPPSSSLQP GKMSDVSPVVAAQQQQQQQQQQQQQQQQQQQ QQQQEAAAAAAAAAAAAAAAAAAVPRLRPPHDN RTMVEIIADHPAELVRTDSPNFLCSVLPSHWRCNK TLPVAFKVVALGEVPDGTVVTVMAGNDENYSAE LRNASAVMKNQVARFNDLRFVGRSGRGKSFTLTI

SEQ ID NO:	Description	Sequence
		TVFTNPPQVATYHRAIKVTVDGPREPRRHRQKLD DSKPSLFSDRLSDLGRIPHPSMRVGVPPQNPRPSL NSAPSPFNPQGQSQITDPRQAQSSPPWSYDQSYPS YLSQMTSPSIHSTTPLSSTRGTGLPAITDVPRRISD DDTATSDFCLWPSTLSKKSQAGASELGPFSDPRQF PSISSLTESRFSNPRMHYPATFTYTPPVTSGMSLG MSATTHYHTYLPPPYPGSSQSQSGPFQTSSTPYLY YGTSSGSYQFPMVPGGDRSPSRMLPPCTTTSNGST LLNPNLPNQNDGVDADGSHSSSPTVLNSSGRMDE SVWRPY
64	Human Alp protein	MISPFLVLAIGTCLTNSLVPEKEKDPKYWRDQAQ ETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTY NTNAQVPDSAGTATAYLCGVKANEGTVGVSAAT ERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTR VNHATPSAAYAHSADRDWYSDNEMPPEALSQGC KDIAYQLMHNIRDIDVIMGGGRKYMYPKNKTDV EYESDEKARGTRLDGLDLVDTWKSFKPRYKHSHF IWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRN NVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRID HGHHEGKAKQALHEAVEMDRAIGQAGSLTSSED TLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDT DKKPFTAILYGNGPGYKVVGGERENVSMVDYAH NNYQAQSAVPLRHETHGGEDVAVFSKGPMAHLL HGVHEQNYVPHVMAYAACIGANLGHCAPASSAG SLAAGPLLLALALYPLSVLF
65	Human Osterix protein	MASSLLEEEVHYGSSPLAMLTAACSKFGGSSPLR DSTTLGKAGTKKPYSVGSDLSASKTMGDAYPAPF TSTNGLLSPAGSPPAPTSGYANDYPPFSHSFPGPTG TQDPGLLVPKGHSSSDCLPSVYTSLDMTHPYGSW YKAGIHAGISPGPGNTPTPWWDMHPGGNWLGGG QGQGDGLQGTLPTGPAQPPLNPQLPTYPSDFAPL NPAPYPAPHLLQPGPQHVLPQDVYKPKAVGNSG QLEGSGGAKPPRGASTGGSGGYGGSGAGRSSCDC PNCQELERLGAAAAGLRKKPIHSCHIPGCGKVYG KASHLKAHLRWHTGERPFVCNWLFCGKRFTRSD ELERHVRTHTREKKFTCLLCSKRFTRSDHLSKHQ RTHGEPGPGPPPSGPKELGEGRSTGEEEASQTPRP SASPATPEKAPGGSPEQSNLLEI
66	Human Klotho protein	MPASAPPRRPRPPPPSLSLLLVLLGLGGRRLRAEP GDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWA VGSAAYQTEGGWQQHGKGASIWDTFTHHPLAPP GDSRNASLPLGAPSPLQPATGDVASDSYNNVFRD TEALRELGVTHYRFSISWARVLPNGSAGVPNREG LRYYRRLLERLRELGVQPVVTLYHWDLPQRLQD

SEQ ID NO:	Description	Sequence
		AYGGWANRALADHFRDYAELCFRHFGGQVKYW ITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVA HNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSH WINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDG DYPESMKNNLSSILPDFTESEKKFIKGTADFFALCF GPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFN HPQIFIVENGWFVSGTTKRDDAKYMYYLKKFIME TLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRR GLFYVDFLSQDKMLLPKSSALFYQKLIEKNGFPPL PENQPLEGTFPCDFAWGVVDNYIQVDTTLSQFTD LNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFA AIQPQIALLQEMHVTHFRFSLDWALILPLGNQSQV NHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLLARQGAWENPYTALAFAEYARLCFQEL GHHVKLWITMNEPYTRNMTYSAGHNLLKAHALA WHVYNEKFRHAQNGKISIALQADWIEPACPFSQK DKEVAERVLEFDIGWLAEPIFGSGDYPWVMRDW LNQRNNFLLPYFTEDEKKLIQGTFDFLALSHYTTIL VDSEKEDPIKYNDYLEVQEMTDITWLNSPSQVAV VPWGLRKVLNWLKFKYGDLPMYIISNGIDDGLHA EDDQLRVYYMQNYINEALKAHILDGINLCGYFAY SFNDRTAPRFGLYRYAADQFEPKASMKHYRKIID SNGFPGPETLERFCPEEFTVCTECSFFHTRKSLLAF IAFLFFASIISLSLIFYYSKKGRRSYK
67	Human Sm22-alpha protein	MANKGPSYGMSREVQSKIEKKYDEELEERLVEWI IVQCGPDVGRPDRGRLGFQVWLKNGVILSKLVNS LYPDGSKPVKVPENPPSMVFKQMEQVAQFLKAA EDYGVIKTDMFQTVDLFEGKDMAAVQRTLMALG SLAVTKNDGHYRGDPNWFMKKAQEHKREFTESQ LQEGKHVIGLQMGSNRGASQAGMTGYGRPRQIIS
68	Human alpha-SMA protein	MCEEEDSTALVCDNGSGLCKAGFAGDDAPRAVF PSIVGRPRHQGVMVGMGQKDSYVGDEAQSKRGI LTLKYPIEHGIITNWDDMEKIWHHSFYNELRVAPE EHPTLLTEAPLNPKANREKMTQIMFETFNVPAMY VAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEG YALPHAIMRLDLAGRDLTDYLMKILTERGYSFVT TAEREIVRDIKEKLCYVALDFENEMATAASSSSLE KSYELPDGQVITIGNERFRCPETLFQPSFIGMESAG IHETTYNSIMKCDIDIRKDLYANNVLSGGTTMYPG IADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGS ILASLSTFQQMWISKQEYDEAGPSIVHRKCF
69	Human MYOCD protein	MTLLGSEHSLLIRSKFRSVLQLRLQQRRTQEQLAN QGIIPPLKRPAEFHEQRKHLDSDKAKNSLKRKARN RCNSADLVNMHILQASTAERSIPTAQMKLKRARL ADDLNEKIALRPGPLELVEKNILPVDSAVKEAIKG

SEQ ID NO:	Description	Sequence
		NQVSFSKSTDAFAFEEDSSSDGLSPDQTRSEDPQN SAGSPPDAKASDTPSTGSLGTNQDLASGSENDRN DSASQPSHQSDAGKQGLGPPSTPIAVHAAVKSKS LGDSKNRHKKPKDPKPKVKKLKYHQYIPPDQKA EKSPPPMDSAYARLLQQQQLFLQLQILSQQQQQQ QHRFSYLGMHQAQLKEPNEQMVRNPNSSSTPLSN TPLSPVKNSFSGQTGVSSFKPGPLPPNLDDLKVSE LRQQLRIRGLPVSGTKTALMDRLRPFQDCSGNPV PNFGDITTVTFPVTPNTLPNYQSSSSTSALSNGFYH FGSTSSSPPISPASSDLSVAGSLPDTFNDASPSFGLH PSPVHVCTEESLMSSLNGGSVPSELDGLDSEKDK MLVEKQKVINELTWKLQQEQRQVEELRMQLQKQ KRNNCSEKKPLPFLAASIKQEEAVSSCPFASQVPV KRQSSSSECHPPACEAAQLQPLGNAHCVESSDQT NVLSSTFLSPQCSPQHSPLGAVKSPQHISLPPSPNN PHFLPSSSGAQGEGHRVSSPISSQVCTAQNSGAHD GHPPSFSPHSSSLHPPFSGAQADSSHGAGGNPCPK SPCVQQKMAGLHSSDKVGPKFSIPSPTFSKSSSAIS EVTQPPSYEDAVKQQMTRSQQMDELLDVLIESGE MPADAREDHSCLQKVPKIPRSSRSPTAVLTKPSAS FEQASSGSQIPFDPYATDSDEHLEVLLNSQSPLGK MSDVTLLKIGSEEPHFDGIMDGFSGKAAEDLFNA HEILPGPLSPMQTQFSPSSVDSNGLQLSFTESPWET MEWLDLTPPNSTPGFSALTTSSPSIFNIDFLDVTDL NLNSSMDLHLQQW
70	Human alpha-SMA mRNA	CTCTCCCCGCCCCCGCGGGGCGGCGCGCACTCA CCCACCCGCGCCGGAGCGGACCTTTGGCTTGGC TTGTCAGGGCTTGTCCAGGAGTTCCGCTCCTCTC TCCAACCGGGGTCCCCCTCCAGCGACCCTAAAG CTTCCCAGACTTCCGCTTCAATTCCTGTCCGCAC CCCACGCCCACCTCAACGTGGAGCGCAGTGGTC TCCGAGGAGCGCCGGAGCTGCCCCGCCTGCCCA GCGGGGTCAGCACTTCGCATCAAGGCCCAAGA AAAGCAAGTCCTCCAGCGTTCTGAGCACCCGGG CCTGAGGGAAGGTCCTAACAGCCCCCGGGAGC CAGTCTCCAACGCCTCCCGCAGCAGCCCGCCGC TCCCAGGTGCCCGCGTGCGCCGCTGCCGCCGCA ATCCCGCACGCGTCCCGCGCCCGCCCCACTTTG CCTATCCCCGGGACTAAGACGGGAATCCTGTGA AGCAGCTCCAGCTATGTGTGAAGAAGAGGACA GCACTGCCTTGGTGTGTGACAATGGCTCTGGGC TCTGTAAGGCCGGCTTTGCTGGGGACGATGCTC CCAGGGCTGTTTTCCCATCCATTGTGGGACGTC CCAGACATCAGGGGGTGATGGTGGGAATGGGA CAAAAAGACAGCTACGTGGGTGACGAAGCACA

SEQ ID NO:	Description	Sequence
		GAGCAAAAGAGGAATCCTGACCCTGAAGTACC CGATAGAACATGGCATCATCACCAACTGGGAC GACATGGAAAAGATCTGGCACCACTCTTTCTAC AATGAGCTTCGTGTTGCCCCTGAAGAGCATCCC ACCCTGCTCACGGAGGCACCCCTGAACCCCAAG GCCAACCGGGAGAAAATGACTCAAATTATGTTT GAGACTTTCAATGTCCCAGCCATGTATGTGGCT ATCCAGGCGGTGCTGTCTCTCTATGCCTCTGGA CGCACAACTGGCATCGTGCTGGACTCTGGAGAT GGTGTCACCCACAATGTCCCCATCTATGAGGGC TATGCCTTGCCCCATGCCATCATGCGTCTGGAT CTGGCTGGCCGAGATCTCACTGACTACCTCATG AAGATCCTGACTGAGCGTGGCTATTCCTTCGTT ACTACTGCTGAGCGTGAGATTGTCCGGGACATC AAGGAGAAACTGTGTTATGTAGCTCTGGACTTT GAAAATGAGATGGCCACTGCCGCATCCTCATCC TCCCTTGAGAAGAGTTACGAGTTGCCTGATGGG CAAGTGATCACCATCGGAAATGAACGTTTCCGC TGCCCAGAGACCCTGTTCCAGCCATCCTTCATC GGGATGGAGTCTGCTGGCATCCATGAAACCACC TACAACAGCATCATGAAGTGTGATATTGACATC AGGAAGGACCTCTATGCTAACAATGTCCTATCA GGGGGCACCACTATGTACCCTGGCATTGCCGAC CGAATGCAGAAGGAGATCACGGCCCTAGCACC CAGCACCATGAAGATCAAGATCATTGCCCCTCC GGAGCGCAAATACTCTGTCTGGATCGGTGGCTC CATCCTGGCCTCTCTGTCCACCTTCCAGCAGAT GTGGATCAGCAAACAGGAATACGATGAAGCCG GGCCTTCCATTGTCCACCGCAAATGCTTCTAAA ACACTTTCCTGCTCCTCTCTGTCTCTAGCACACA ACTGTGAATGTCCTGTGGAATTATGCCTTCAGT TCTTTTCCAAATCATTCCTAGCCAAAGCTCTGAC TCGTTACCTATGTGTTTTTTAATAAATCTGAAAT AGGCTACTGGTAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAA
71	Human MYOCD mRNA	AATCGCCGGCAGCCTATGACATCAGACAGGAA CGCCTGGGATGCCGCGCTGCTCCTGGCCAACCT CCGAGGAGGAGGAGGGTCCCGCCGGCTAAGAG TTAATTAGCCCCGCACGGCGAGGGGGGAGGCG CCAGTTTTCTGGGGACACTGGCTGCCACTGTAC TCCTACCCAGGGGAGCTCACGGAGAGTTGGATG AATTCTGGGTTGTTAGCTGCGGTCAGCTGGGCT CCCGGGAGCCTGTTGCTGGTGGAGAACAGGGG GCGCCTGGCCAAGGGACCAGCGGCTTGCTGAG ACTCAACATGACACTCCTGGGGTCTGAGCATTC

SEQ ID NO:	Description	Sequence
		CTTGCTGATTAGGAGCAAGTTCAGATCAGTTTT ACAGTTAAGACTTCAACAAAGAAGGACCCAGG AACAACTGGCTAACCAAGGCATAATACCACCA CTGAAACGTCCAGCTGAATTCCATGAGCAAAGA AAACATTTGGATAGTGACAAGGCTAAAAATTCC CTGAAGCGCAAAGCCAGAAACAGGTGCAACAG TGCCGACTTGGTTAATATGCACATACTCCAAGC TTCCACTGCAGAGAGGTCCATTCCAACTGCTCA GATGAAGCTGAAAAGAGCCCGACTCGCCGATG ATCTCAATGAAAAAATTGCTCTACGACCAGGGC CACTGGAGCTGGTGGAAAAAAACATTCTTCCTG TGGATTCTGCTGTGAAAGAGGCCATAAAAGGTA ACCAGGTGAGTTTCTCCAAATCCACGGATGCTT TTGCCTTTGAAGAGGACAGCAGCAGCGATGGG CTTTCTCCGGATCAGACTCGAAGTGAAGACCCC CAAAACTCAGCGGGATCCCCGCCAGACGCTAA AGCCTCAGATACCCCTTCGACAGGTTCTCTGGG GACAAACCAGGATCTTGCTTCTGGCTCAGAAAA TGACAGAAATGACTCAGCCTCACAGCCCAGCCA CCAGTCAGATGCGGGGAAGCAGGGGCTTGGCC CCCCCAGCACCCCCATAGCCGTGCATGCTGCTG TAAAGTCCAAATCCTTGGGTGACAGTAAGAACC GCCACAAAAAGCCCAAGGACCCCAAGCCAAAG GTGAAGAAGCTTAAATATCACCAGTACATTCCC CCAGACCAGAAGGCAGAGAAGTCCCCTCCACC TATGGACTCAGCCTACGCTCGGCTGCTCCAGCA ACAGCAGCTGTTCCTGCAGCTCCAAATCCTCAG CCAGCAGCAGCAGCAGCAGCAACACCGATTCA GCTACCTAGGGATGCACCAAGCTCAGCTTAAGG AACCAAATGAACAGATGGTCAGAAATCCAAAC TCTTCTTCAACGCCACTGAGCAATACCCCCTTGT CTCCTGTCAAAAACAGTTTTTCTGGACAAACTG GTGTCTCTTCTTTCAAACCAGGCCCACTCCCACC TAACCTGGATGATCTGAAGGTCTCTGAATTAAG ACAACAGCTTCGAATTCGGGGCTTGCCTGTGTC AGGCACCAAAACGGCTCTCATGGACCGGCTTCG ACCCTTCCAGGACTGCTCTGGCAACCCAGTGCC GAACTTTGGGGATATAACGACTGTCACTTTTCC TGTCACACCCAACACGCTGCCCAATTACCAGTC TTCCTCTTCTACCAGTGCCCTGTCCAACGGCTTC TACCACTTTGGCAGCACCAGCTCCAGCCCCCCG ATCTCCCCAGCCTCCTCTGACCTGTCAGTCGCTG GGTCCCTGCCGGACACCTTCAATGATGCCTCCC CCTCCTTCGGCCTGCACCCGTCCCCAGTCCACG TGTGCACGGAGGAAAGTCTCATGAGCAGCCTG

SEQ ID NO:	Description	Sequence
		AATGGGGGCTCTGTTCCTTCTGAGCTGGATGGG CTGGACTCCGAGAAGGACAAGATGCTGGTGGA GAAGCAGAAGGTGATCAATGAACTCACCTGGA AACTCCAGCAAGAGCAGAGGCAGGTGGAGGAG CTGAGGATGCAGCTTCAGAAGCAGAAAAGGAA TAACTGTTCAGAGAAGAAGCCGCTGCCTTTCCT GGCTGCCTCCATCAAGCAGGAAGAGGCTGTCTC CAGCTGTCCTTTTGCATCCCAAGTACCTGTGAA AAGACAAAGCAGCAGCTCAGAGTGTCACCCAC CGGCTTGTGAAGCTGCTCAACTCCAGCCTCTTG GAAATGCTCATTGTGTGGAGTCCTCAGATCAAA CCAATGTACTTTCTTCCACATTTCTCAGCCCCCA GTGTTCCCCTCAGCATTCACCGCTGGGGGCTGT GAAAAGCCCACAGCACATCAGTTTGCCCCCATC ACCCAACAACCCTCACTTTCTGCCCTCATCCTCC GGGGCCCAGGGAGAAGGGCACAGGGTCTCCTC GCCCATCAGCAGCCAGGTGTGCACTGCACAGA ACTCAGGAGCACACGATGGCCATCCTCCAAGCT TCTCTCCCCATTCTTCCAGCCTCCACCCGCCCTT CTCTGGAGCCCAAGCAGACAGCAGTCATGGTGC CGGGGGAAACCCTTGTCCCAAAAGCCCATGTGT ACAGCAAAAGATGGCTGGTTTACACTCTTCTGA TAAGGTGGGGCCAAAGTTTTCAATTCCATCCCC AACTTTTTCTAAGTCAAGTTCAGCAATTTCAGA GGTAACACAGCCTCCATCCTATGAAGATGCCGT AAAGCAGCAAATGACCCGGAGTCAGCAGATGG ATGAACTCCTGGACGTGCTTATTGAAAGCGGAG AAATGCCAGCAGACGCTAGAGAGGATCACTCA TGTCTTCAAAAAGTCCCAAAGATACCCAGATCT TCCCGAAGTCCAACTGCTGTCCTCACCAAGCCC TCGGCTTCCTTTGAACAAGCCTCTTCAGGCAGC CAGATCCCCTTTGATCCCTATGCCACCGACAGT GATGAGCATCTTGAAGTCTTATTAAATTCCCAG AGCCCCCTAGGAAAGATGAGTGATGTCACCCTT CTAAAAATTGGGAGCGAAGAGCCTCACTTTGAT GGGATAATGGATGGATTCTCTGGGAAGGCTGCA GAAGACCTCTTCAATGCACATGAGATCTTGCCA GGCCCCCTCTCTCCAATGCAGACACAGTTTTCA CCCTCTTCTGTGGACAGCAATGGGCTGCAGTTA AGCTTCACTGAATCTCCCTGGGAAACCATGGAG TGGCTGGACCTCACTCCGCCAAATTCCACACCA GGCTTTAGCGCCCTCACCACCAGCAGCCCCAGC ATCTTCAACATCGATTTCCTGGATGTCACTGATC TCAATTTGAATTCTTCCATGGACCTTCACTTGCA GCAGTGGTAGAATGCCCAATGCACCAGTGCTAT

SEQ ID NO:	Description	Sequence
		GGAAGACCAATGGAGTTCCATGGGGGAAAGCA CACAGCCATACATACTTTACTGTCCAAAAACAG AAGAAGAAGAAGAGAATTAAAAAGAAGCAATG ATTTCTGTGCCAATGAACAAGAACAAAAGTCAT TTTTAGAAATACATATACTGTAATATTTACCAA CAGTCAGTAACTGTTAATGATTTCAACAATGCA TTAAAAGAATGTGCTTTCTCAGATTAAGGATGC CAAAAAAGATATTTCACTGCCTTTTCAAAGACC AGTATATTTTCTAGCCCATAATTTTTCTCAGGCA TTGTTGGGGCATAAGCTCACACTGTAAGCTTTT CTCATGAATTCACTAGACATAACGTGGAAGGAA AACGTAGTCTTTTGGGAGTACAGGGAAGCCAGC CCCTCAAAGCTTATGGAAGACATACCTGCAATG GAAGCTGTTGCCCAATGTCTCCATTACTATCTTT CAAAAGAGAAGCCAGACCCAGCTTCAGATCAA AAGTTCTTGAGACAGAGGAACAAAACCAATCG ATTTCCAGGGAAGCTAATCAACTCTCTTTTCCCT CTACCACAAAACTGCCCTGCTGGAGTGGTTCTG AACCTGTACCCAGGACTCGATGTGGTCACTAAT AACAATTAACCTGAACTGAGTCCACAGAACTCC ACTCGGAACTTTCTTCTTTTTTAACTAGTGGCCC AATCATTCCCACCATCTCTGTGCTGATAAGTAC GTGTCCTAGATGAGAACCCTGAAGAATGCAGA CCTTCTTCCCCCGAAGGAGATGCCACAAGCTCT CCAACACAGCCCCCTTTAGTTCCAAAGACTAGA GATGACCACATTGGTAGAAGTATATCTCGAGGC ACAGGAAGGGAGCCCCACCAGGGATAATTCAG ACAGGACTAGAGAATAACATCATTTCACATACC CTGGGATAAACACCCTGGGTTCCTATAGAAGGA CTATTACTTATGGGAGTCCAACTTCTCCTTTTGT TTTGTTATTATCAGTTTATCTTTCTCCCACTCCA CTTTTCCTTCAAGGTACCAATCCTTTCCTGTTCC TCGTTTGGCCATCTTTCTTTTTCTGCCTCCACAT TGGGAGGGGAGGACTTCTCAGTTCTAACAAGCT GCCATACTCCTAAGAAAGCCATTTTTGAAAAAT TTAACAATCCAGGTTCTTCTGGAGAACTCATTC TCCACACGCACAGTTTGCTGCAAAAGGAAGTTG CAAGAATTTCTTGAGGAAGAAACTGGTGACTTG GTCCATCAGTCACGAAGTTCTTTCTATTCTCGTT TAGTTTTCAAGAAATTATTGGTTTGTGTTGCTCT GGGGAAATTGGAAATCATTACATTGTAAAGAC AAATATGGATGATATTTACAAGAGAGAATTTCA GATCTGGGTTTTTGAAAGAAAACAGAATTGCGC ATTGAAAACGATGGAAGGAAAAAGACAATGGT CTAATGTGCATTCCTCATTACCTCTCGTGGCTTT

SEQ ID NO:	Description	Sequence
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SEQ ID NO:	Description	Sequence
		TGACAAATCAGGCATGACTAAAAGATGTACAG AGACTTACGAAGATGGTCACATTCAAGTTCCCT AATGCTCTTAGAACCTGAAGATGACCATGTGTA GTTTTCTTAAGACCTCTGAACCCCCATGGTGAT GAAGACTTGAAGACATTTGCAGCTATCTGCTGC AGTCTGGTAGATTCATACTTATCTAAAGAAGTC AAAAAATTTATTCGTGCAAGTGCTTGCAGGAAG CCAGTGCTTATTAGTAGTGACCCTGCTTCTATCA ACGTTATTGAGACAACACATATTCTATTCTAAG GGAGAAAGAGGGAGGAAGAGAGGGAGGGAGG GAGGAAGAAGAGGGAGGGAGCGAGGAAGGAA GATAGGAGATGGGTAGGGGGGTAAAGAGAAAA GGAGGGAGAAGGGAAGGAAGGAAAGAAGAGA GGAAAGAAAGGAGGGAAGGAAAAAAGGGCCA AACTTTCTGATCTATGAACTTCTCAGTTCAGCTG TCACATTATGAGAAGTAAATCAGAATTTTTTTA AGGAGAAGTCATTCTTAGCACTACAATAATTGT ACCAGTAATTGAGGAAACCAAGACAATCTTCAC CTGAATAATAGAGGGTCTGAGAACTGTCAGCCT TTTGCCATTCAAAAACATTTATGTCCAACCTGA AAAAAAAGCATCAATAAAACCTATCCCAAGCA TTCAAAATAGTCCTTTCCAAATGTTATTTATTTT AAAGTCAATCAGCTCTTTTAGAAACAGATTCTG GTCTGGCTGAAAACTCCCACAACAAATTTACTC ATCCAGTGGCTAATATTTAATGCCCACCATGGG CAGAGCACACAAATCTTCAGATAAACAATACTG AATTGATTCAGCACAGATTGTTCAGATTTGAAT GACCAGGGAGTTGTATTTGCACATGCAAGAACA CTAAGAATCTCCCAGTCCTCAAACTAGAAACCT TTCAGCTACGATGAAAAAAAAAAAGGGGTCTT CATTTTTCCAAGAGGGGGTGGAGGTGGGGATCA CTTTTTAGCTAAAAGCTATCTCTCACTTCAAAAT TCTTGTCTTTTTCTTTGTGGACAAACACCAGTAG TCTATCACTTGGAGATCTTTTAATATCTCCCATC ATTTAAAACATCCACGAGAGTTTGAAGATTTGT GTTGATTGCCAGATACAGAAGCCCCTTGAAAAT AAGGAAAGGGTGGAGGAAGCATTTTTGTGTCCT ATCCCTACTTATCGTAGCAGCTCTATAGACAAA AGGGACACTTACTGGTGAGCCTCTGGCCCTTAA AAGAAAATCATCTAAGAATATGAAGGCAATTT GATTTCCCCCCACAGCCCTCAGCTGCCTTCCTC ACAGAAGGAAGTTCCCAAAATTGCTGGTACAC AGTTTGCAATCAAATATCAGATATGAGAAAACC TGTAGTGAAGAGTCTGGGTTCTTGGTTTTCTCAT AAATCCAATATAAATTTGTAGGTTGGTTCAGGG

SEQ ID NO:	Description	Sequence
		TCAAAATTGCCAGTGCTTTATTAGACAGATGAT ACTGATAGACACACAGAGCCCAGGTCCTGGAA CAAGACAATCCTGTAGTGCCAAGATCTGGTCAG TTGCGTTAAGGAGCTGGGTTTGATTCTAGAGTC CAGGTTTATAGAGAAACCCTGGCTAGATTGAGC CTACCCATGGGGAGACGATTTCAAGACAGGAT GAGATCTGGGAAGAATTTTGTTGTCATCTGCCA GGGAAATTATCACAGGACTCATTGAATGCAATA ACATGTGAGTAAGTTCCCTTTTGATTCTGGGAA TCAGCGATTTTCCCTGTGGATTAAGACAAACCA ACGCCAGAAGGTCTCCTGTGCTTATTTTAACCA TCTGCTCCCATCGTGAACCCTGGAGCATGCATT TCCTAGAAGTGGTTTCATAGCTCCTGTGTGTTCA TGGAAAAGGGGAGTATAATGATGGGGATGCTG GAAGCTTTTTTAATGTTTTCCAAAGGAAAGGAA CCCACACTGCTCCCCAGAGTTCCTTTCCAATGG CCCTGCAGTAAGAACGGAGGACAATGTATTGCT GGGTGCTTAAAATCCTCCCTCAGTGAAGCACAA AGAGACACTTTGTAAAGAAAAAAAGAGCAAGC ATAGGTTCTCTGTGGGACCTTGTGGAGTGGTGT TTTCACGTTGGTCTCTTTGGCTCAATTGAGCATA ATCAGAAAGAAATGTGGGTTATTGGGAAGAGA CAAAAAGCAGTGGCTAAAATACCAAAGTTGGC ATGTGTTCTTTTTTAAAAAAAAAAAAAAATGCA TATATTTTTAAATAAAATGTTTATTTTAAAAAG AAAAAAAAAAAAAAAAAA
72	Human BSAP protein	MISPFLVLAIGTCLTNSLVPEKEKDPKYWRDQAQ ETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTY NTNAQVPDSAGTATAYLCGVKANEGTVGVSAAT ERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTR VNHATPSAAYAHSADRDWYSDNEMPPEALSQGC KDIAYQLMHNIRDIDVIMGGGRKYMYPKNKTDV EYESDEKARGTRLDGLDLVDTWKSFKPRYKHSHF IWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRN NVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRID HGHHEGKAKQALHEAVEMDRAIGQAGSLTSSED TLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDT DKKPFTAILYGNGPGYKVVGGERENVSMVDYAH NNYQAQSAVPLRHETHGGEDVAVFSKGPMAHLL HGVHEQNYVPHVMAYAACIGANLGHCAPASSAG SLAAGPLLLALALYPLSVLF
73	Human BSAP mRNA	CCGGGCCTCACTCGGGCCCCGCGGCCGCCTTTA TAAGGCGGCGGGGGTGGTGGCCCGGGCCGCGT TGCGCTCCCGCCACTCCGCGCCCGCTATCCTGG

SEQ ID NO:	Description	Sequence
		CTCCGTGCTCCCACGCGCTTGTGCCTGGACGGA CCCTCGCCAGTGCTCTGCGCAGGATTGGAACAT CAGTTAACATCTGACCACTGCCAGCCCACCCCC TCCCACCCACGTCGATTGCATCTCTGGGCTCCA GGGATAAAGCAGGTCTTGGGGTGCACCATGATT TCACCATTCTTAGTACTGGCCATTGGCACCTGC CTTACTAACTCCTTAGTGCCAGAGAAAGAGAAA GACCCCAAGTACTGGCGAGACCAAGCGCAAGA GACACTGAAATATGCCCTGGAGCTTCAGAAGCT CAACACCAACGTGGCTAAGAATGTCATCATGTT CCTGGGAGATGGGATGGGTGTCTCCACAGTGAC GGCTGCCCGCATCCTCAAGGGTCAGCTCCACCA CAACCCTGGGGAGGAGACCAGGCTGGAGATGG ACAAGTTCCCCTTCGTGGCCCTCTCCAAGACGT ACAACACCAATGCCCAGGTCCCTGACAGCGCC GGCACCGCCACCGCCTACCTGTGTGGGGTGAAG GCCAATGAGGGCACCGTGGGGGTAAGCGCAGC CACTGAGCGTTCCCGGTGCAACACCACCCAGGG GAACGAGGTCACCTCCATCCTGCGCTGGGCCAA GGACGCTGGGAAATCTGTGGGCATTGTGACCAC CACGAGAGTGAACCATGCCACCCCCAGCGCCG CCTACGCCCACTCGGCTGACCGGGACTGGTACT CAGACAACGAGATGCCCCCTGAGGCCTTGAGCC AGGGCTGTAAGGACATCGCCTACCAGCTCATGC ATAACATCAGGGACATTGACGTGATCATGGGG GGTGGCCGGAAATACATGTACCCCAAGAATAA AACTGATGTGGAGTATGAGAGTGACGAGAAAG CCAGGGGCACGAGGCTGGACGGCCTGGACCTC GTTGACACCTGGAAGAGCTTCAAACCGAGATAC AAGCACTCCCACTTCATCTGGAACCGCACGGAA CTCCTGACCCTTGACCCCCACAATGTGGACTAC CTATTGGGTCTCTTCGAGCCAGGGGACATGCAG TACGAGCTGAACAGGAACAACGTGACGGACCC GTCACTCTCCGAGATGGTGGTGGTGGCCATCCA GATCCTGCGGAAGAACCCCAAAGGCTTCTTCTT GCTGGTGGAAGGAGGCAGAATTGACCACGGGC ACCATGAAGGAAAAGCCAAGCAGGCCCTGCAT GAGGCGGTGGAGATGGACCGGGCCATCGGGCA GGCAGGCAGCTTGACCTCCTCGGAAGACACTCT GACCGTGGTCACTGCGGACCATTCCCACGTCTT CACATTTGGTGGATACACCCCCCGTGGCAACTC TATCTTTGGTCTGGCCCCCATGCTGAGTGACAC AGACAAGAAGCCCTTCACTGCCATCCTGTATGG CAATGGGCCTGGCTACAAGGTGGTGGGCGGTG AACGAGAGAATGTCTCCATGGTGGACTATGCTC

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		ACAACAACTACCAGGCGCAGTCTGCTGTGCCCC TGCGCCACGAGACCCACGGCGGGGAGGACGTG GCCGTCTTCTCCAAGGGCCCCATGGCGCACCTG CTGCACGGCGTCCACGAGCAGAACTACGTCCCC CACGTGATGGCGTATGCAGCCTGCATCGGGGCC AACCTCGGCCACTGTGCTCCTGCCAGCTCGGCA GGCAGCCTTGCTGCAGGCCCCCTGCTGCTCGCG CTGGCCCTCTACCCCCTGAGCGTCCTGTTCTGA GGGCCCAGGGCCCGGGCACCCACAAGCCCGTG ACAGATGCCAACTTCCCACACGGCAGCCCCCCC CTCAAGGGGCAGGGAGGTGGGGGCCTCCTCAG CCTCTGCAACTGCAAGAAAGGGGACCCAAGAA ACCAAAGTCTGCCGCCCACCTCGCTCCCCTCTG GAATCTTCCCCAAGGGCCAAACCCACTTCTGGC CTCCAGCCTTTGCTCCCTCCCCGCTGCCCTTTGG CCAACAGGGTAGATTTCTCTTGGGCAGGCAGAG AGTACAGACTGCAGACATTCTCAAAGCCTCTTA TTTTTCTAGCGAACGTATTTCTCCAGACCCAGA GGCCCTGAAGCCTCCGTGGAACATTCTGGATCT GACCCTCCCAGTCTCATCTCCTGACCCTCCCACT CCCATCTCCTTACCTCTGGAACCCCCCAGGCCC TACAATGCTCATGTCCCTGTCCCCAGGCCCAGC CCTCCTTCAGGGGAGTTGAGGTCTTTCTCCTCA GGACAAGGCCTTGCTCACTCACTCACTCCAAGA CCACCAGGGTCCCAGGAAGCCGGTGCCTGGGT GGCCATCCTACCCAGCGTGGCCCAGGCCGGGA AGAGCCACCTGGCAGGGCTCACACTCCTGGGCT CTGAACACACACGCCAGCTCCTCTCTGAAGCGA CTCTCCTGTTTGGAACGGCAAAAAAAAATTTTT TTTTCTCTTTTTGGTGGTGGTTAAAAGGGAACA CAAAACATTTAAATAAAACTTTCCAAATATTTC CGAGGACAAAAAAAAAAA
74	Snail mRNA	ATTCATTGCGCCGCGGCACGGCCTAGCGAGTGG TTCTTCTGCGCTACTGCTGCGCGAATCGGCGAC CCCAGTGCCTCGACCACTATGCCGCGCTCTTTC CTCGTCAGGAAGCCCTCCGACCCCAATCGGAAG CCTAACTACAGCGAGCTGCAGGACTCTAATCCA GAGTTTACCTTCCAGCAGCCCTACGACCAGGCC CACCTGCTGGCAGCCATCCCACCTCCGGAGATC CTCAACCCCACCGCCTCGCTGCCAATGCTCATC TGGGACTCTGTCCTGGCGCCCCAAGCCCAGCCA ATTGCCTGGGCCTCCCTTCGGCTCCAGGAGAGT CCCAGGGTGGCAGAGCTGACCTCCCTGTCAGAT GAGGACAGTGGGAAAGGCTCCCAGCCCCCCAG CCCACCCTCACCGGCTCCTTCGTCCTTCTCCTCT

SEQ ID NO:	Description	Sequence
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75	Dkk1 mRNA	GCAGAGCTCTGTGCTCCCTGCAGTCAGGACTCT GGGACCGCAGGGGGCTCCCGGACCCTGACTCTG CAGCCGAACCGGCACGGTTTCGTGGGGACCCA GGCTTGCAAAGTGACGGTCATTTTCTCTTTCTTT CTCCCTCTTGAGTCCTTCTGAGATGATGGCTCTG

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		GGCGCAGCGGGAGCTACCCGGGTCTTTGTCGCG ATGGTAGCGGCGGCTCTCGGCGGCCACCCTCTG CTGGGAGTGAGCGCCACCTTGAACTCGGTTCTC AATTCCAACGCTATCAAGAACCTGCCCCCACCG CTGGGCGGCGCTGCGGGGCACCCAGGCTCTGCA GTCAGCGCCGCGCCGGGAATCCTGTACCCGGGC GGGAATAAGTACCAGACCATTGACAACTACCA GCCGTACCCGTGCGCAGAGGACGAGGAGTGCG GCACTGATGAGTACTGCGCTAGTCCCACCCGCG GAGGGGACGCAGGCGTGCAAATCTGTCTCGCCT GCAGGAAGCGCCGAAAACGCTGCATGCGTCAC GCTATGTGCTGCCCCGGGAATTACTGCAAAAAT GGAATATGTGTGTCTTCTGATCAAAATCATTTC CGAGGAGAAATTGAGGAAACCATCACTGAAAG CTTTGGTAATGATCATAGCACCTTGGATGGGTA TTCCAGAAGAACCACCTTGTCTTCAAAAATGTA TCACACCAAAGGACAAGAAGGTTCTGTTTGTCT CCGGTCATCAGACTGTGCCTCAGGATTGTGTTG TGCTAGACACTTCTGGTCCAAGATCTGTAAACC TGTCCTGAAAGAAGGTCAAGTGTGTACCAAGCA TAGGAGAAAAGGCTCTCATGGACTAGAAATATT CCAGCGTTGTTACTGTGGAGAAGGTCTGTCTTG CCGGATACAGAAAGATCACCATCAAGCCAGTA ATTCTTCTAGGCTTCACACTTGTCAGAGACACT AAACCAGCTATCCAAATGCAGTGAACTCCTTTT ATATAATAGATGCTATGAAAACCTTTTATGACC TTCATCAACTCAATCCTAAGGATATACAAGTTC TGTGGTTTCAGTTAAGCATTCCAATAACACCTT CCAAAAACCTGGAGTGTAAGAGCTTTGTTTCTT TATGGAACTCCCCTGTGATTGCAGTAAATTACT GTATTGTAAATTCTCAGTGTGGCACTTACCTGT AAATGCAATGAAACTTTTAATTATTTTTCTAAA GGTGCTGCACTGCCTATTTTTCCTCTTGTTATGT AAATTTTTGTACACATTGATTGTTATCTTGACTG ACAAATATTCTATATTGAACTGAAGTAAATCAT TTCAGCTTATAGTTCTTAAAAGCATAACCCTTTA CCCCATTTAATTCTAGAGTCTAGAACGCAAGGA TCTCTTGGAATGACAAATGATAGGTACCTAAAA TGTAACATGAAAATACTAGCTTATTTTCTGAAA TGTACTATCTTAATGCTTAAATTATATTTCCCTT TAGGCTGTGATAGTTTTTGAAATAAAATTTAAC ATTTAATATCATGAAATGTTATAAGTAGACATA CATTTTGGGATTGTGATCTTAGAGGTTTGTGTGT GTGTACGTATGTGTGTGTTCTACAAGAACGGAA GTGTGATATGTTTAAAGATGATCAGAGAAAAG

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76	Col1a1 mRNA	TCGTCGGAGCAGACGGGAGTTTCTCCTCGGGGT CGGAGCAGGAGGCACGCGGAGTGTGAGGCCAC GCATGAGCGGACGCTAACCCCCTCCCCAGCCAC AAAGAGTCTACATGTCTAGGGTCTAGACATGTT CAGCTTTGTGGACCTCCGGCTCCTGCTCCTCTTA GCGGCCACCGCCCTCCTGACGCACGGCCAAGA GGAAGGCCAAGTCGAGGGCCAAGACGAAGACA TCCCACCAATCACCTGCGTACAGAACGGCCTCA GGTACCATGACCGAGACGTGTGGAAACCCGAG CCCTGCCGGATCTGCGTCTGCGACAACGGCAAG GTGTTGTGCGATGACGTGATCTGTGACGAGACC AAGAACTGCCCCGGCGCCGAAGTCCCCGAGGG CGAGTGCTGTCCCGTCTGCCCCGACGGCTCAGA GTCACCCACCGACCAAGAAACCACCGGCGTCG AGGGACCCAAGGGAGACACTGGCCCCCGAGGC CCAAGGGGACCCGCAGGCCCCCCTGGCCGAGA TGGCATCCCTGGACAGCCTGGACTTCCCGGACC CCCCGGACCCCCCGGACCTCCCGGACCCCCTGG CCTCGGAGGAAACTTTGCTCCCCAGCTGTCTTA TGGCTATGATGAGAAATCAACCGGAGGAATTTC CGTGCCTGGCCCCATGGGTCCCTCTGGTCCTCG TGGTCTCCCTGGCCCCCCCTGGTGCACCTGGTCC CCAAGGCTTCCAAGGTCCCCCTGGTGAGCCTGG CGAGCCTGGAGCTTCAGGTCCCATGGGTCCCCG AGGTCCCCCAGGTCCCCCTGGAAAGAATGGAG ATGATGGGGAAGCTGGAAAACCTGGTCGTCCTG GTGAGCGTGGGCCTCCTGGGCCTCAGGGTGCTC GAGGATTGCCCGGAACAGCTGGCCTCCCTGGAA TGAAGGGACACAGAGGTTTCAGTGGTTTGGATG GTGCCAAGGGAGATGCTGGTCCTGCTGGTCCTA AGGGTGAGCCTGGCAGCCCTGGTGAAAATGGA GCTCCTGGTCAGATGGGCCCCCGTGGCCTGCCT GGTGAGAGAGGTCGCCCTGGAGCCCCTGGCCCT GCTGGTGCTCGTGGAAATGATGGTGCTACTGGT GCTGCCGGGCCCCCTGGTCCCACCGGCCCCGCT GGTCCTCCTGGCTTCCCTGGTGCTGTTGGTGCTA AGGGTGAAGCTGGTCCCCAAGGGCCCCGAGGC TCTGAAGGTCCCCAGGGTGTGCGTGGTGAGCCT GGCCCCCCTGGCCCTGCTGGTGCTGCTGGCCCT GCTGGAAACCCTGGTGCTGATGGACAGCCTGGT

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		GCTAAAGGTGCCAATGGTGCTCCTGGTATTGCT GGTGCTCCTGGCTTCCCTGGTGCCCGAGGCCCC TCTGGACCCCAGGGCCCCGGCGGCCCTCCTGGT CCCAAGGGTAACAGCGGTGAACCTGGTGCTCCT GGCAGCAAAGGAGACACTGGTGCTAAGGGAGA GCCTGGCCCTGTTGGTGTTCAAGGACCCCCTGG CCCTGCTGGAGAGGAAGGAAAGCGAGGAGCTC GAGGTGAACCCGGACCCACTGGCCTGCCCGGA CCCCCTGGCGAGCGTGGTGGACCTGGTAGCCGT GGTTTCCCTGGCGCAGATGGTGTTGCTGGTCCC AAGGGTCCCGCTGGTGAACGTGGTTCTCCTGGC CCTGCTGGCCCCAAAGGATCTCCTGGTGAAGCT GGTCGTCCCGGTGAAGCTGGTCTGCCTGGTGCC AAGGGTCTGACTGGAAGCCCTGGCAGCCCTGGT CCTGATGGCAAAACTGGCCCCCCTGGTCCCGCC GGTCAAGATGGTCGCCCCGGACCCCCAGGCCC ACCTGGTGCCCGTGGTCAGGCTGGTGTGATGGG ATTCCCTGGACCTAAAGGTGCTGCTGGAGAGCC CGGCAAGGCTGGAGAGCGAGGTGTTCCCGGAC CCCCTGGCGCTGTCGGTCCTGCTGGCAAAGATG GAGAGGCTGGAGCTCAGGGACCCCCTGGCCCT GCTGGTCCCGCTGGCGAGAGAGGTGAACAAGG CCCTGCTGGCTCCCCCGGATTCCAGGGTCTCCC TGGTCCTGCTGGTCCTCCAGGTGAAGCAGGCAA ACCTGGTGAACAGGGTGTTCCTGGAGACCTTGG CGCCCCTGGCCCCTCTGGAGCAAGAGGCGAGA GAGGTTTCCCTGGCGAGCGTGGTGTGCAAGGTC CCCCTGGTCCTGCTGGTCCCCGAGGGGCCAACG GTGCTCCCGGCAACGATGGTGCTAAGGGTGATG CTGGTGCCCCTGGAGCTCCCGGTAGCCAGGGCG CCCCTGGCCTTCAGGGAATGCCTGGTGAACGTG GTGCAGCTGGTCTTCCAGGGCCTAAGGGTGACA GAGGTGATGCTGGTCCCAAAGGTGCTGATGGCT CTCCTGGCAAAGATGGCGTCCGTGGTCTGACTG GCCCCATTGGTCCTCCTGGCCCTGCTGGTGCCC CTGGTGACAAGGGTGAAAGTGGTCCCAGCGGC CCTGCTGGTCCCACTGGAGCTCGTGGTGCCCCC GGAGACCGTGGTGAGCCTGGTCCCCCCGGCCCT GCTGGCTTTGCTGGCCCCCCTGGTGCTGACGGC CAACCTGGTGCTAAAGGCGAACCTGGTGATGCT GGTGCTAAAGGCGATGCTGGTCCCCCTGGCCCT GCCGGACCCGCTGGACCCCCTGGCCCCATTGGT AATGTTGGTGCTCCTGGAGCCAAAGGTGCTCGC GGCAGCGCTGGTCCCCCTGGTGCTACTGGTTTC CCTGGTGCTGCTGGCCGAGTCGGTCCTCCTGGC

SEQ ID NO:	Description	Sequence
		CCCTCTGGAAATGCTGGACCCCCTGGCCCTCCT GGTCCTGCTGGCAAAGAAGGCGGCAAAGGTCC CCGTGGTGAGACTGGCCCTGCTGGACGTCCTGG TGAAGTTGGTCCCCCTGGTCCCCCTGGCCCTGC TGGCGAGAAAGGATCCCCTGGTGCTGATGGTCC TGCTGGTGCTCCTGGTACTCCCGGGCCTCAAGG TATTGCTGGACAGCGTGGTGTGGTCGGCCTGCC TGGTCAGAGAGGAGAGAGAGGCTTCCCTGGTCT TCCTGGCCCCTCTGGTGAACCTGGCAAACAAGG TCCCTCTGGAGCAAGTGGTGAACGTGGTCCCCC TGGTCCCATGGGCCCCCCTGGATTGGCTGGACC CCCTGGTGAATCTGGACGTGAGGGGGCTCCTGG TGCCGAAGGTTCCCCTGGACGAGACGGTTCTCC TGGCGCCAAGGGTGACCGTGGTGAGACCGGCC CCGCTGGACCCCCTGGTGCTCCTGGTGCTCCTG GTGCCCCTGGCCCCGTTGGCCCTGCTGGCAAGA GTGGTGATCGTGGTGAGACTGGTCCTGCTGGTC CCGCCGGTCCTGTCGGCCCTGTTGGCGCCCGTG GCCCCGCCGGACCCCAAGGCCCCCGTGGTGAC AAGGGTGAGACAGGCGAACAGGGCGACAGAGG CATAAAGGGTCACCGTGGCTTCTCTGGCCTCCA GGGTCCCCCTGGCCCTCCTGGCTCTCCTGGTGA ACAAGGTCCCTCTGGAGCCTCTGGTCCTGCTGG TCCCCGAGGTCCCCCTGGCTCTGCTGGTGCTCCT GGCAAAGATGGACTCAACGGTCTCCCTGGCCCC ATTGGGCCCCCTGGTCCTCGCGGTCGCACTGGT GATGCTGGTCCTGTTGGTCCCCCCGGCCCTCCT GGACCTCCTGGTCCCCCTGGTCCTCCCAGCGCT GGTTTCGACTTCAGCTTCCTGCCCCAGCCACCTC AAGAGAAGGCTCACGATGGTGGCCGCTACTAC CGGGCTGATGATGCCAATGTGGTTCGTGACCGT GACCTCGAGGTGGACACCACCCTCAAGAGCCTG AGCCAGCAGATCGAGAACATCCGGAGCCCAGA GGGCAGCCGCAAGAACCCCGCCCGCACCTGCC GTGACCTCAAGATGTGCCACTCTGACTGGAAGA GTGGAGAGTACTGGATTGACCCCAACCAAGGCT GCAACCTGGATGCCATCAAAGTCTTCTGCAACA TGGAGACTGGTGAGACCTGCGTGTACCCCACTC AGCCCAGTGTGGCCCAGAAGAACTGGTACATC AGCAAGAACCCCAAGGACAAGAGGCATGTCTG GTTCGGCGAGAGCATGACCGATGGATTCCAGTT CGAGTATGGCGGCCAGGGCTCCGACCCTGCCGA TGTGGCCATCCAGCTGACCTTCCTGCGCCTGAT GTCCACCGAGGCCTCCCAGAACATCACCTACCA CTGCAAGAACAGCGTGGCCTACATGGACCAGC

SEQ ID NO:	Description	Sequence
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SEQ ID NO:	Description	Sequence
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77	Activin A mRNA	ATGCCCTTGCTTTGGCTGAGAGGATTTCTGTTG GCAAGTTGCTGGATTATAGTGAGGAGTTCCCCC ACCCCAGGATCCGAGGGGCACAGCGCGGCCCC CGACTGTCCGTCCTGTGCGCTGGCCGCCCTCCC AAAGGATGTACCCAACTCTCAGCCAGAGATGGT GGAGGCCGTCAAGAAGCACATTTTAAACATGCT GCACTTGAAGAAGAGACCCGATGTCACCCAGC CGGTACCCAAGGCGGCGCTTCTGAACGCGATCA GAAAGCTTCATGTGGGCAAAGTCGGGGAGAAC GGGTATGTGGAGATAGAGGATGACATTGGAAG GAGGGCAGAAATGAATGAACTTATGGAGCAGA CCTCGGAGATCATCACGTTTGCCGAGTCAGGTT GGTGCTGGCATTGGCAGGGGGTGGGGAGGGGT GGGGGGTGGGAGGGTAAAATATATTTCTTTGAC AGTCCCAGGAGGAACTTCTTTTCCCTTCAGCTG GAAACTGCCTGGGAAGGTTATTAGTTATTAGGT GATGGTAGCGGACTAGCCGACGGAGGGCAGGC AGGGGAGGGGGAGAGGACTTTACAGAAAAGGA ATTCTCGGTCGAGCTCTGCCTGGAGATGACTGG CTTACACTTACTAAACCCAGCGGGTCACACAGA GAGGAAGCTCGGGGCCAATGTTGAGCTGGAAG GCAGACTGTGAGGGGCTGCCTTGCCCTGCCTGT GAAACAGATCTGAGCAGCCGGAGGAAAGCCGC GGCATTTTCGGGTGCTAGGGGAGCAGAGGAGG CTTCCGGACCCCATCCAAGTTTTTATTGAGGGT AGAGGGGTGAATGTACCAGGATTGGAGTGGAA TGGCACAGATGAAGTCACTCTCTTAAAACAAAC CTTCCCCTTTAAAAGTCCAATCTGGGGCCACAT TGGAGAAGCAGGGCATATTTATGAGTGACAGTC ATTTTTACCTTTAGAAAATGTCTATAAGTGCAC AGGCACCACATTCAAGACAGGGAAGAGCTACT TTGGGGGACAGTTGTCATTGAACCAGCAGTTAC TTTTGGGACACTGACTTTTGCTCTCTGAAAGAA AAAAAAATAAATAAAACAACCAGTTTTGTTCTT TCTAAAGTTACTAAGAGCTCTCTGCCAAGGAAC GAACCTTGACAAAGTACTCTCAGATACTACGCT GAAGTCACTCAATCTTAAGAGGAAGAAG
78	Snail primer 1	TCGGAAGCCTAACTACAGCGA
79	Snail primer 2	AGATGAGCATTGGCAGCGAG

SEQ ID NO:	Description	Sequence
80	Dkk1 primer 1	CCTTGAACTCGGTTCTCAATTCC
81	Dkk1 primer 2	CAATGGTCTGGTACTTATTCCCG
82	Col1a1 primer 1	GAGGGCCAAGACGAAGACATC
83	Col1a1 primer 2	CAGATCACGTCATCGCACAAC
84	Activin primer 1	TCATCACGTTTGCCGAGTCA
85	Activin primer 2	GCTAGTCCGCTACCATCACC
86	Human Axin2 mRNA	ACTGGCTCCGCGAGCCTGGCCCGGGGGAGTCG GCTGGAGCCGGCTGCGCTTTGATAAGGTCCTGG CAACTCAGTAACAGCCCGAGAGCCGGGAAATA AAAATAACCCCTCAGAGCGATGGATTTCGGGGC CGCCCGGCGGCCGAGGCGCCCGCCGAAGGCCC TGCTGTAAAAGAGAGGAGGTTCAGATGAGCCC CTGCTGACTTGAGAGAGACAGAGAGACCACGC CGATTGCTGAGAGGAACTGGAAGAAGAAAAAT TCCCAGACTCAGTGGGAAGAGCTCCCTCACCAT GAGTAGCGCTATGTTGGTGACTTGCCTCCCGGA CCCCAGCAGCAGCTTCCGTGAGGATGCCCCGCG GCCCCCAGTGCCAGGGGAAGAAGGGGAGACCC CACCGTGTCAGCCAGGGGTGGGCAAGGGCCAG GTCACCAAACCCATGCCTGTCTCTTCCAACACC AGGCGGAACGAAGATGGGTTGGGGGAGCCGGA GGGGCGGGCATCTCCGGATTCCCCTCTGACCCG GTGGACCAAGTCCTTACACTCCTTATTGGGCGA TCAAGACGGTGCTTACCTGTTCCGAACTTTCCT GGAGAGGGAGAAATGCGTGGATACCTTAGACT TCTGGTTTGCCTGCAATGGATTCAGGCAGATGA ACCTGAAGGATACCAAAACTTTACGAGTAGCCA AAGCGATCTACAAAAGGTACATTGAGAACAAC AGCATTGTCTCCAAGCAGCTGAAGCCTGCCACC AAGACCTACATAAGAGATGGCATCAAGAAGCA GCAGATTGATTCCATCATGTTTGACCAGGCGCA GACCGAGATCCAGTCGGTGATGGAGGAAAATG CCTACCAGATGTTTTTGACTTCTGATATATACCT CGAATATGTGAGGAGTGGGGGAGAAAACACAG CTTACATGAGTAATGGGGGACTCGGGAGCCTAA AGGTCGTGTGTGGCTATCTCCCCACCTTGAATG AAGAAGAGGAGTGGACTTGTGCCGACTTCAAG TGCAAACTTTCGCCAACCGTGGTTGGCTTGTCC AGCAAAACTCTGAGGGCCACGGCGAGTGTGAG GTCCACGGAAACTGTTGACAGTGGATACAGGTC CTTCAAGAGGAGCGATCCTGTTAATCCTTATCA CATAGGTTCTGGCTATGTCTTTGCACCAGCCAC CAGCGCCAACGACAGTGAGATATCCAGTGATG CGCTGACGGATGATTCCATGTCCATGACGGACA GCAGTGTAGATGGAATTCCTCCTTATCGTGTGG

SEQ ID NO:	Description	Sequence
		GCAGTAAGAAACAGCTCCAGAGAGAAATGCAT CGCAGTGTGAAGGCCAATGGCCAAGTGTCTCTA CCTCATTTCCCGAGAACCCACCGCCTGCCCAAG GAGATGACCCCCGTGGAACCCGCCACCTTTGCA GCTGAGCTGATCTCGAGGCTGGAAAAGCTGAA GCTGGAGTTGGAGAGCCGCCACAGCCTGGAGG AGCGCCTGCAGCAGATCCGAGAGGATGAAGAG AGAGAGGGCTCCGAGCTCACACTCAATTCGCGG GAGGGGGCGCCCACGCAGCACCCCCTCTCCCTA CTGCCCTCCGGCAGCTACGAGGAAGACCCGCA GACGATACTGGACGATCACCTGTCCAGGGTCCT CAAGACCCCTGGCTGCCAGTCTCCAGGCGTAGG CCGCTATAGCCCCCGCTCCCGCTCCCCGGACCA CCACCACCACCACCATTCGCAGTACCACTCCCT GCTCCCGCCCCGGTGGCAAGCTGCCTCCCGCGGC CGCCTCGCCGGGCGCCTGCCCCCTCCTCGGGGG CAAAGGCTTTGTGACCAAGCAGACGACGAAGC ATGTCCACCACCACTACATCCACCACCATGCCG TCCCCAAGACCAAGGAGGAGATCGAGGCGGAG GCCACGCAGCGGGTGCACTGCTTCTGCCCTGGG GGCAGCGAGTATTACTGCTACTCGAAATGCAAA AGCCACTCCAAGGCTCCGGAAACCATGCCCAG CGAGCAGTTTGGCGGCAGCAGAGGCAGTACCTT GCCCAAACGCAATGGGAAAGGCACGGAGCCGG GCCTGGCCCTGCCCGCCAGGGAAGGAGGGGCC CCCGGCGGAGCTGGGGCCCTGCAGCTTCCCCGG GAGGAAGGAGACAGGTCGCAGGATGTCTGGCA GTGGATGCTGGAGAGTGAGCGGCAGAGCAAGC CCAAGCCCCATAGTGCCCAAAGCACAAAAAAG GCCTACCCCTTGGAGTCTGCCCGCTCGTCTCCA GGCGAACGAGCCAGCCGGCACCATCTGTGGGG GGGCAACAGCGGGCACCCCCGCACCACCCCCC GTGCCCACCTGTTCACCCAGGACCCTGCGATGC CTCCCCTGACCCCACCCAACACGCTGGCTCAGC TGGAGGAGGCCTGTCGCAGGCTAGCTGAGGTGT CGAAGCCCCCAAAGCAGCGGTGCTGTGTGGCC AGTCAGCAGAGGGACAGGAATCATTCGGCCAC TGTTCAGACGGGAGCCACACCCTTCTCCAATCC AAGCCTGGCTCCAGAAGATCACAAAGAGCCAA AGAAACTGGCAGGTGTCCACGCGCTCCAGGCC AGTGAGTTGGTTGTCACTTACTTTTTCTGTGGGG AAGAAATTCCATACCGGAGGATGCTGAAGGCT CAGAGCTTGACCCTGGGCCACTTTAAAGAGCAG CTCAGCAAAAAGGGAAATTATAGGTATTACTTC AAAAAAGCAAGCGATGAGTTTGCCTGTGGAGC

SEQ ID NO:	Description	Sequence
		GGTGTTTGAGGAGATCTGGGAGGATGAGACGG TGCTCCCGATGTATGAAGGCCGGATTCTGGGCA AAGTGGAGCGGATCGATTGAGCCCTGGGGTCTG GCTTTGGTGAACTGTTGGAGCCCGAAGCTCTTG TGAACTGTCTTGGCTGTGAGCAACTGCGACAAA ACATTTTGAAGGAAAATTAAACCAATGAAGAA GACAAAGTCTAAGGAAGAATCGGCCAGTGGGC CTTCGGGAGGGCGGGGGGAGGTTGATTTTCATG ATTCATGAGCTGGGTACTGACTGAGATAAGAAA AGCCTGAACTATTTATTAAAAACATGACCACTC TTGGCTATTGAAGATGCTGCCTGTATTTGAGAG ACTGCCATACATAATATATGACTTCCTAGGGAT CTGAAATCCATAAACTAAGAGAAACTGTGTATA GCTTACCTGAACAGGAATCCTTACTGATATTTA TAGAACAGTTGATTTCCCCCATCCCCAGTTTAT GGATATGCTGCTTTAAACTTGGAAGGGGGAGAC AGGAAGTTTTAATTGTTCTGACTAAACTTAGGA GTTGAGCTAGGAGTGCGTTCATGGTTTCTTCAC TAACAGAGGAATTATGCTTTGCACTACGTCCCT CCAAGTGAAGACAGACTGTTTTAGACAGACTTT TTAAAATGGTGCCCTACCATTGACACATGCAGA AATTGGTGCGTTTTGTTTTTTTTTTTCCTATGCTG CTCTGTTTTGTCTTAAAGGTCTTGAGGGTTGACC ATGTTGCGTCATCATCAACATTTTGGGGGTTGT GTTGGATGGGATGATCTGTTGCAGAGGGAGAG GCAGGGAACCCTGCTCCTTCGGGCCCCAGGTTG ATCCTGTGACTGAGGCTCCCCCTCATGTAGCCT CCCCAGGCCCAGGGCCCTGAGGCCTGCTAGAAT CACTGCCGCTGTGCTTTCGTGGAAATGACAGTT CCTTGTTTTTTTTGTTTCTGTTTTTGTTTTACATT AGTCATTGGACCACAGCCATTCAGGAACTACCC CCTGCCCCACAAAGAAATGAACAGTTGTAGGG AGACCCAGCAGCACCTTTCCTCCACACACCTTC ATTTTGATGTTCGGGTTTTTGTGTTAAGTTAATC TGTACATTCTGTTTGCCATTGTTACTTGTACTAT ACATCTGTATATAGTGTACGGCAAAAGAGTATT AATCCACTATCTCTAGTGCTTGACTTTAAATCA GTACAGTACCTGTACCTGCACGGTCACCCGCTC CGTGTGTCGCCCTATATTGAGGGCTCAAGCTTT CCCTTGTTTTTTTGAAAGGGGTTTATGTATAAATA TATTTTATGCCTTTTTATTACAAGTCTTGTACTC AATGACTTTTGTCATGACATTTTGTTCTACTTAT ACTGTAAATTATGCATTATAAAGAGTTCATTTA AGGAAAATTACTTGGTACAATAATTATTGTAAT TAAGAGATGTAGCCTTTATTAAAATTTTATATTT

SEQ ID NO:	Description	Sequence
		TTCAAAAAAAAAAAA
87	Human Axin2 amino acid sequence	MSSAMLVTCLPDPSSSFREDAPRPPVPGEEGETPP CQPGVGKGQVTKPMPVSSNTRRNEDGLGEPEGR ASPDSPLTRWTKSLHSLLGDQDGAYLFRTFLERE KCVDTLDFWFACNGFRQMNLKDTKTLRVAKAIY KRYIENNSIVSKQLKPATKTYIRDGIKKQQIDSIMF DQAQTEIQSVMEENAYQMFLTSDIYLEYVRSGGE NTAYMSNGGLGSLKVVCGYLPTLNEEEEWTCAD FKCKLSPTVVGLSSKTLRATASVRSTETVDSGYRS FKRSDPVNPYHIGSGYVFAPATSANDSEISSDALT DDSMSMTDSSVDGIPPYRVGSKKQLQREMHRSV KANGQVSLPHFPRTHRLPKEMTPVEPATFAAELIS RLEKLKLELESRHSLEERLQQIREDEEREGSELTLN SREGAPTQHPLSLLPSGSYEEDPQTILDDHLSRVL KTPGCQSPGVGRYSPRSRSPDHHHHHHSQYHSLL PPGGKLPPAAASPGACPLLGGKGFVTKQTTKHVH HHYIHHHAVPKTKEEIEAEATQRVHCFCPGGSEY YCYSKCKSHSKAPETMPSEQFGGSRGSTLPKRNG KGTEPGLALPAREGGAPGGAGALQLPREEGDRSQ DVWQWMLESERQSKPKPHSAQSTKKAYPLESAR SSPGERASRHHLWGGNSGHPRTTPRAHLFTQDPA MPPLTPPNTLAQLEEACRRLAEVSKPPKQRCCVA SQQRDRNHSATVQTGATPFSNPSLAPEDHKEPKK LAGVHALQASELVVTYFFCGEEIPYRRMLKAQSL TLGHFKEQLSKKGNYRYYFKKASDEFACGAVFEE IWEDETVLPMYEGRILGKVERID

11. EQUIVALENTS

[00560] Although the invention is described in detail with reference to specific embodiments thereof, it will be understood that variations which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00561] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference in their entireties.

WHAT IS CLAIMED:

1. A method for treating and/or preventing cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an activin receptor type II (ActRII) signaling inhibitor, wherein the subject has:

(a) elevated levels of runt-related transcription factor 2 (Runx2) as compared to levels of Runx2 in a reference population;

(b) elevated levels of alkaline phosphatase (Alp) as compared to levels of Alp in a reference population;

(c) elevated levels of snail homolog 1 (Snail) as compared to levels of Snail in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of dickkopf homolog 1 (Dkk1) as compared to levels of Dkk1 in a reference population;

(f) elevated levels of collagen type 1 alpha 1 (col1a1) as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of bone-specific alkaline phosphatase (BSAP) as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha smooth muscle actin (alpha-SMA) as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of myocardin (MYOCD) as compared to levels of MYOCD in a reference population;

(n) decreased levels of smooth muscle protein 22-alpha (Sm22-alpha) as compared to levels of Sm22-alpha in a reference population

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

2. A method for treating and/or preventing vascular calcification in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor, wherein the subject has:

(a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population;

(b) elevated levels of Alp as compared to levels of Alp in a reference population;

(c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population;

(f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of BSAP as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population;

(n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population;

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(p) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

3. A method for treating and/or preventing cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor, wherein the subject has:

(a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population;

(b) elevated levels of Alp as compared to levels of Alp in a reference population;

(c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population;

(f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of BSAP as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; and/or

(n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population;

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population

(p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population; and

wherein the cardiovascular disease is associated with and/or results from vascular calcification.

4. A method for treating and/or preventing elevated levels of arterial stiffness in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor, wherein the subject has:

(a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population;

(b) elevated levels of Alp as compared to levels of Alp in a reference population;

(c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population;

(f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of BSAP as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; and/or

(n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population;

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

5. A method for treating and/or preventing cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor, wherein the subject has:

(a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population;

(b) elevated levels of Alp as compared to levels of Alp in a reference population;

(c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population;

(f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of BSAP as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; and/or

(n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population;

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population,

wherein the cardiovascular disease is associated with and/or results from elevated levels of arterial stiffness.

6. A method for treating and/or preventing left ventricular hypertrophy (LVH) in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor, wherein the subject has:

(a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population;

(b) elevated levels of Alp as compared to levels of Alp in a reference population;

(c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population;

(f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of BSAP as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; and/or

(n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population;

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

7. A method for treating and/or preventing cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor, wherein the subject has:

(a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population;

(b) elevated levels of Alp as compared to levels of Alp in a reference population;

(c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population;

(f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of BSAP as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; and/or

(n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population;

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population;

wherein the cardiovascular disease is associated with and/or results from left ventricular hypertrophy.

8. A method for treating and/or preventing cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor, wherein the subject has:

(a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population;

(b) elevated levels of Alp as compared to levels of Alp in a reference population;

(c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population;

(f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of BSAP as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population; and/or

(n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population;

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population; and

wherein the cardiovascular disease is associated with and/or results from renal disease.

9. A method for reducing bone resorption in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor, wherein the subject has:

(a) elevated levels of Runx2 as compared to levels of Runx2 in a reference population;

(b) elevated levels of Alp as compared to levels of Alp in a reference population;

(c) elevated levels of Snai1 as compared to levels of Snai1 in a reference population;

(d) elevated levels of phosphosmad2 as compared to levels of phosphosmad2 in a reference population;

(e) elevated levels of Dkk1 as compared to levels of Dkk1 in a reference population;

(f) elevated levels of col1a1 as compared to levels of col1a1 in a reference population;

(g) elevated levels of activin as compared to levels of activin in a reference population;

(h) elevated levels of BSAP as compared to levels of BSAP in a reference population;

(i) elevated levels of CTX as compared to levels of CTX in a reference population;

(j) elevated levels of Osterix as compared to levels of Osterix in a reference population;

(k) decreased levels of Klotho as compared to levels of Klotho in a reference population;

(l) decreased levels of alpha-SMA as compared to levels of alpha-SMA in a reference population;

(m) decreased levels of MYOCD as compared to levels of MYOCD in a reference population;

(n) decreased levels of Sm22-alpha as compared to levels of Sm22-alpha in a reference population

(o) elevated levels of phosphosmad3 as compared to levels of phosphosmad3 in a reference population;

(p) elevated levels of urinary protein as compared to levels of urinary protein in a reference population; and/or

(q) decreased levels of ActRIIA as compared to levels of ActRIIA in a reference population.

10. The method of any one of claims 1-9, wherein the pharmaceutically effective dose of the ActRII signaling inhibitor is about 15 mg, about 30 mg, about 45 mg, about 60 mg, about 75 mg, about 90 mg, or about 1 g or about 0.1 mg/kg, about 0.13 mg/kg, about 0.2 mg/kg, about 0.26 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg.

11. The method of any one of claims 1-10, wherein the pharmaceutically effective dose is administered via injection.

12. The method of any one of claims 1-11, wherein the pharmaceutically effective dose is administered (i) once every 14 days; (ii) once every 21 days; (iii) once every 28 days, or (iv) once every 42 days.

13. The method of any one of claims 1-11, wherein the pharmaceutically effective dose is administered continuously and/or indefinitely.

14. The method of any one of claims 1-9, wherein the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix, are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, 100%, 200%, or 500% greater than the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix, respectively, in the reference population.

15. The method of any one of claims 1-9, wherein the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 200%, or 500% greater than the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population.

16. The method of any one of claims 1-9, wherein the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively, in the reference population.

17. The method of any one of claims 1-9, wherein the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

18. A method for treating and/or preventing vascular calcification in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the first measurement is taken prior to the commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said vascular calcification.

19. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the first measurement is taken prior to the commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

20. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the cardiovascular disease is associated with and/or results from vascular calcification, wherein the first measurement is taken prior to the commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

21. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the cardiovascular disease is associated with and/or results from renal disease; and wherein the first measurement is taken prior to the commencement of the

treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

22. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the cardiovascular disease is associated with and/or results from elevated levels of arterial stiffness; and wherein the first measurement is taken prior to the commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

23. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the cardiovascular disease is associated with and/or results from left ventricular hypertrophy; and wherein the first measurement is taken prior to the commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

24. A method for reducing bone resorption in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the first measurement is taken prior to the commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby reducing said bone resorption.

25. A method for treating and/or preventing vascular calcification in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said vascular calcification.

26. A method for treating and/or preventing elevated levels of arterial stiffness in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said elevated levels of arterial stiffness.

27. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the cardiovascular disease is associated with and/or results from elevated levels of arterial stiffness, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

28. A method for treating and/or preventing left ventricular hypertrophy (LVH) in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said LVH.

29. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the cardiovascular disease is associated with and/or results from left ventricular hypertrophy, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

30. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

31. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor;

wherein the cardiovascular disease is associated with and/or results from vascular calcification, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

32. A method for treating and/or preventing cardiovascular disease in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor;

wherein the cardiovascular disease is associated with and/or results from renal disease, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby treating and/or preventing said cardiovascular disease.

33. A method for reducing bone resorption in a subject, wherein the method comprises:

(a) administering to the subject an initial dose of an ActRII signaling inhibitor to the subject;

(b) taking a first measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject;

(c) after a period of time, taking a second measurement of the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the subject; and

(d) administering to the subject an adjusted dose of the activin receptor type II signaling inhibitor, and wherein the adjusted dose is based on the detected change between the first measurement and the second measurement, thereby reducing said bone resorption.

34. The method of any one of claims 18-33, wherein the initial dose of the ActRII signaling inhibitor is about 15 mg, about 30 mg, about 45 mg, about 60 mg, about 75 mg, about 90 mg, or about 1 g or about 0.1 mg/kg, about 0.13 mg/kg, about 0.2 mg/kg, about 0.26 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, or about 1.5 mg/kg.

35. The method of any one of claims 18-34, wherein the initial dose is administered via injection.

36. The method of any one of claims 18-35, wherein the initial dose is administered (i) once every 14 days; (ii) once every 21 days, (iii) once every 28 days; or (iv) once every 42 days.

37. The method of any one of claims 18-35, wherein the adjusted dose of the ActRII signaling inhibitor is greater than the initial dose if:

(a) the level of Runx2 is elevated as compared to the level of Runx2 in a reference population;

(b) the level of Alp is elevated as compared to the level of Alp in a reference population;

(c) the level of Snail is elevated as compared to the level of Snail in a reference population;

(d) the level of phosphosmad2 is elevated as compared to the level of phosphosmad2 in a reference population;

(e) the level of Dkk1 is elevated as compared to the level of Dkk1 in a reference population;

(f) the level of col1a1 is elevated as compared to the level of col1a1 in a reference population;

(g) the level of activin is elevated as compared to the level of activin in a reference population;

(h) the level of BSAP is elevated as compared to the level of BSAP in a reference population;

(i) the level of CTX is elevated as compared to the level of CTX in a reference population;

(j) the level of Osterix is elevated as compared to the level of Osterix in a reference population;

(k) the level of Klotho is decreased as compared to the level Klotho in a reference population;

(l) the level of alpha-SMA is decreased as compared to the level of alpha-SMA in a reference population;

(m) the level of MYOCD is decreased as compared to the level of MYOCD in a reference population;

(n) the level of Sm22-alpha is decreased as compared to the level of Sm22-alpha in a reference population;

(o) the level of phosphosmad3 is elevated as compared to the level of phosphosmad3 in a reference population;

(p) the level of urinary protein is elevated as compared to the level of urinary protein in a reference population;

(q) the level of ActRIIA is decreased compared to the level of ActRIIA in a reference population; and/or

(r) the level of Axin2 is decreased compared to the level of Axin2 in a reference population.

38. The method of claim 37, wherein the adjusted dose is about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, or about 35 mg greater than the initial dose, or about 0.05 mg/kg, about 0.1 mg/kg, about 0.15 mg/kg, about 0.25 mg/kg, about 0.3 mg/kg, about 0.35 mg/kg, about 0.4 mg/kg, or about 0.5 mg/kg greater than the initial dose.

39. The method of claim 37 or claim 38, wherein the adjusted dose is administered more frequently than the initial dose.

40. The method of any one of claims 18-39, wherein the adjusted dose is administered every 5, 10, 15, 20, 25, 28, 30, 35, or 40 days.

41. The method of any one of claims 18-33, wherein the adjusted dose of the ActRII signaling inhibitor is less than the initial dose if:

(a) the level of Runx2 is decreased as compared to the level of Runx2 in a reference population;

(b) the level of Alp is decreased as compared to the level of Alp in a reference population;

(c) the level of Snail is decreased as compared to the level of Snail in a reference population;

(d) the level of phosphosmad2 is decreased as compared to the level of phosphosmad2 in a reference population;

(e) the level of Dkk1 is decreased as compared to the level of Dkk1 in a reference population;

(f) the level of col1a1 is decreased as compared to the level of col1a1 in a reference population;

(g) the level of activin is decreased as compared to the level of activin in a reference population;

(h) the level of BSAP is decreased as compared to the level of BSAP in a reference population;

(i) the level of CTX is decreased as compared to the level of CTX in a reference population;

(j) the level of Osterix is decreased as compared to the level of Osterix in a reference population;

(k) the level of Klotho is elevated as compared to the level of Klotho in a reference population;

(l) the level of alpha-SMA is elevated as compared to the level of alpha-SMA in a reference population;

(m) the level of Klotho is elevated as compared to the level of MYOCD in a reference population;

(n) the level of Sm22-alpha is elevated as compared to the level of Sm22-alpha in a reference population;

(o) the level of phosphosmad3 is decreased as compared to the level of phosphosmad3 in a reference population; and/or

(p) the level of urinary protein is decreased as compared to the level of urinary protein in a reference population

(q) the level of ActRIIA is elevated as compared to the level of ActRIIA in a reference population; and/or

(r) the level of Axin2 is elevated as compared to the level of Axin2 in a reference population.

42. The method of claim 41, wherein the adjusted dose is about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, or about 35 mg less than the initial dose, or about 0.05 mg/kg, about 0.1 mg/kg, about 0.15 mg/kg, about 0.25 mg/kg, about 0.3 mg/kg, about 0.35 mg/kg, about 0.4 mg/kg, or about 0.5 mg/kg less than the initial dose.

43. The method of claim 41 or 42, wherein the adjusted dose is administered less frequently than the initial dose.

44. The method of any one of claims 41-43, wherein the adjusted dose is administered every 30, 35, 40, 42, 50, 60, 70, 80, or 90 days.

45. The method of any one of claims 18-44, wherein the adjusted dose is administered continuously and/or indefinitely.

46. The method of any one of claims 18-44, wherein the first measurement is taken prior to the commencement of the treatment.

47. The method of any one of claims 18-44, wherein the first measurement is taken immediately after commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 3 weeks, 4 weeks, or 2 months thereof.

48. The method of any one of claims 18-44, wherein the second measurement is taken immediately after commencement of the treatment or within at most 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months thereof.

49. The method of claim 37, wherein

(a) the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, 100%, 200%, or 500% greater than the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix, respectively, in the reference population; and/or

(b) the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, or 100% less than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in a reference population.

50. The method of claim 49, wherein

(a) the elevated levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix are equal to or about

10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 200%, or 500% greater than the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population; and/or

(b) the decreased levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

51. The method of claim 41, wherein

(a) the elevated levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, 100%, 200%, or 500% greater than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively, in the reference population; and/or

(b) the decreased levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix are about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80% 90%, or 100% less than the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix, respectively, in a reference population.

52. The method of claim 51, wherein

(a) the elevated levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 200%, or 500% greater than the levels of Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha in the top 10%, top 5%, top 4%, top 3%, top 2%, or top 1% in the reference population; and/or

(b) the decreased levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, and/or Osterix are equal to or about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% less than the levels of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp,

BSAP, CTX, and/or Osterix in the bottom 10%, bottom 5%, bottom 4%, bottom 3%, bottom 2%, or bottom 1% in the reference population.

53. The method of any one of claims 1-17, wherein the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is the protein level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively.

54. The method of any one of claims 18-52, wherein the level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is the protein level of Snai1, phosphosmad2, phosphosmad3, urinary protein, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively.

55. The method of claim 53 or 54, wherein the protein level is determined by enzyme-linked immunosorbent assay (ELISA).

56. The method of claim 55, wherein the ELISA is performed with

(a) Runx2-specific antibody SC-390715 (Santa Cruz) to determine Runx2 levels;

(b) Alp-specific antibody SC-98652 (Santa Cruz) to determine Alp levels;

(c) Snai1-specific antibody sc-393172 (Santa Cruz) to determine Snai1 levels;

(d) Phosphosmad2-specific antibody sc-101801 (Santa Cruz) to determine phosphosmad2 levels;

(e) Phosphosmad3-specific antibody sc-130218 (Santa Cruz) to determine phosphosmad2 levels;

(f) Dkk1-specific antibody sc-374574 (Santa Cruz) to determine Dkk1 levels;

(g) Col1a1-specific antibody sc-8784 (Santa Cruz) to determine col1a1 levels;

(h) activin-specific antibody A1594 (Sigma Aldrich) to determine activin levels;

(i) BSAP-specific antibody SC-98652 (Santa Cruz) to determine BSAP levels;

(j) CTX-specific antibody ABIN1173415 (Antibodies Online) to determine CTX levels;

(k) Osterix-specific antibody SC-22538 (Santa Cruz) to determine Osterix levels;

(l) Klotho-specific antibody SC-22218 (Santa Cruz) to determine Klotho levels;

(m) Alpha-SMA-specific antibody SC-53142 (Santa Cruz) to determine alpha-SMA levels;

(n) MYOCD-specific antibody SC-21561 (Santa Cruz) to determine MYOCD levels; and/or

(o) Sm22-alpha-specific antibody SC-271719 (Santa Cruz) to determine Sm22-alpha levels

(p) ActRIIA-specific antibody ab135634 (Abcam) to determine ActRIIA levels.

57. The method of any one claims 1-17, wherein the level of Snai1, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha is the mRNA level of Snai1, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, and/or Sm22-alpha, respectively.

58. The method of any one claims 18-52, wherein the level of Snai1, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha is the mRNA level of Snai1, Dkk1, col1a1, activin, Runx2, Alp, BSAP, CTX, Osterix, Klotho, alpha-SMA, MYOCD, ActRIIA, Axin2, and/or Sm22-alpha, respectively.

59. The method of claim 57 or 58, wherein the mRNA level is determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

60. The method of claim 59, wherein the qRT-PCR is performed with

(a) Runx2-specific primers (SEQ ID NOS: 48 and 49) to determine Runx2 levels;

(b) Alp-specific primers (SEQ ID NOS: 50 and 51) to determine Alp levels;

(c) Col1a1-specific primers (SEQ ID NOS: 82 and 83) to determine col1a1 levels;

(d) Snai1-specific primers (SEQ ID NOS:78 and 79) to determine Snai1 levels;

(e) Dkk1-specific primers (SEQ ID NOS: 80 and 81) to determine Dkk1 levels;

(f) Activin-specific primers (SEQ ID NOS: 84 and 85) to determine activin levels;

(g) Osterix-specific primers (SEQ ID NOS: 52 and 53) to determine Osterix levels;

(h) Klotho-specific primers (SEQ ID NOS: 54 and 55) to determine Klotho levels; and/or

(i) Sm22-alpha-specific primers (SEQ ID NOS: 56 and 57) to determine Sm22-alpha levels.

61. The method of any one of claims 1-60, wherein the levels are in a tissue.

62. The method of claim 61, wherein the tissue is aorta, serum, bone marrow, liver, or spleen.

63. The method of any one of claims 2, 3, 18, or 25, wherein the vascular calcification is calcific atherosclerosis, calcific medial vasculopathy (also known as Mönckeberg's medial calcific sclerosis), medial calcification, elastocalcinosis, calcific uremic arterolopathy, calcific aortic valvular stenosis, or portal vein calcification.

64. The method of claim 1, 19, or 26, wherein the cardiovascular disease is a disease associated with vascular calcification such as atherosclerosis, hyperlipidemia, osteoporosis, hypertension, inflammation, type 2 diabetes mellitus, end-stage renal disease, required

amputation, pseudoxanthoma elasticum, congenital bicuspid valve, rheumatic heart disease, portal hypertension, or liver disease.

65. The method of claim 1, 19, or 26, wherein the cardiovascular disease is secondary to chronic kidney disease.

66. The method of claim 65, wherein the chronic kidney disease is stage 3, 4, or 5 chronic kidney disease.

67. The method of claim 65, wherein the chronic kidney disease is chronic kidney disease mineral and bone disease.

68. The method of any one of claims 1-67, wherein the ActRII signaling inhibitor is a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) 90% identical to SEQ ID NO:2;
(b) 95% identical to SEQ ID NO:2;
(c) 98% identical to SEQ ID NO:2;
(d) SEQ ID NO:2;
(e) 90% identical to SEQ ID NO:3;
(f) 95% identical to SEQ ID NO:3;
(g) 98% identical to SEQ ID NO:3;
(h) SEQ ID NO:3;
(i) 90% identical to SEQ ID NO:6;
(j) 95% identical to SEQ ID NO:6;
(k) 98% identical to SEQ ID NO:6;
(l) SEQ ID NO:6;
(m) 90% identical to SEQ ID NO:7;
(n) 95% identical to SEQ ID NO:7;
(o) 98% identical to SEQ ID NO:7;
(p) SEQ ID NO:7;
(q) 90% identical to SEQ ID NO:12;
(r) 95% identical to SEQ ID NO:12;

(s) 98% identical to SEQ ID NO:12;

(t) SEQ ID NO:12;

(u) 90% identical to SEQ ID NO:17;

(v) 95% identical to SEQ ID NO:17;

(w) 98% identical to SEQ ID NO:17;

(x) SEQ ID NO:17;

(y) 90% identical to SEQ ID NO:20;

(z) 95% identical to SEQ ID NO:20;

(aa) 98% identical to SEQ ID NO:20;

(bb) SEQ ID NO:20;

(cc) 90% identical to SEQ ID NO:21;

(dd) 95% identical to SEQ ID NO:21;

(ee) 98% identical to SEQ ID NO:21; and

(ff) SEQ ID NO:21.

69. The method of any one of claims 1-68, wherein the ActRII signaling inhibitor is a polypeptide comprising the amino acid sequence of SEQ ID NO:7.

70. The method of any one of claims 1-69, wherein the ActRII signaling inhibitor is a humanized fusion-protein consisting of the extracellular domain of ActRIIA and the human IgG1 Fc domain.

71. The method of any one of claims 1-70, wherein the subject is human.

72. The method of any one of claims 1-71, wherein the reference population consists of 1, 5, 10, 25, 50, 75, 100, 200, 250, 300, 400, 500, or 1000 individuals.

73. The method of any one of claims 1-72, wherein the reference population consists of healthy people.

74. The method of any one of claims 1-72, wherein the reference population consists of people of the same age, weight, and/or gender as the subject.

75. The method of any one of claims 1-72, wherein the reference population consists of people without cardiovascular disease.

76. The method of any one of claims 1-72, wherein the reference population consists of people without vascular calcification.

77. The method of any one of claims 1-72, wherein the reference population consists of people without renal disease.

78. The method of any one of claims 1-72, wherein the reference population consists of people without chronic kidney disease.

79. The method of any one of claims 1-72, wherein the reference population consists of people without pathologically elevated levels of arterial stiffness.

80. The method of any one of claims 1-72, wherein the reference population consists of people without left ventricular hypertrophy.

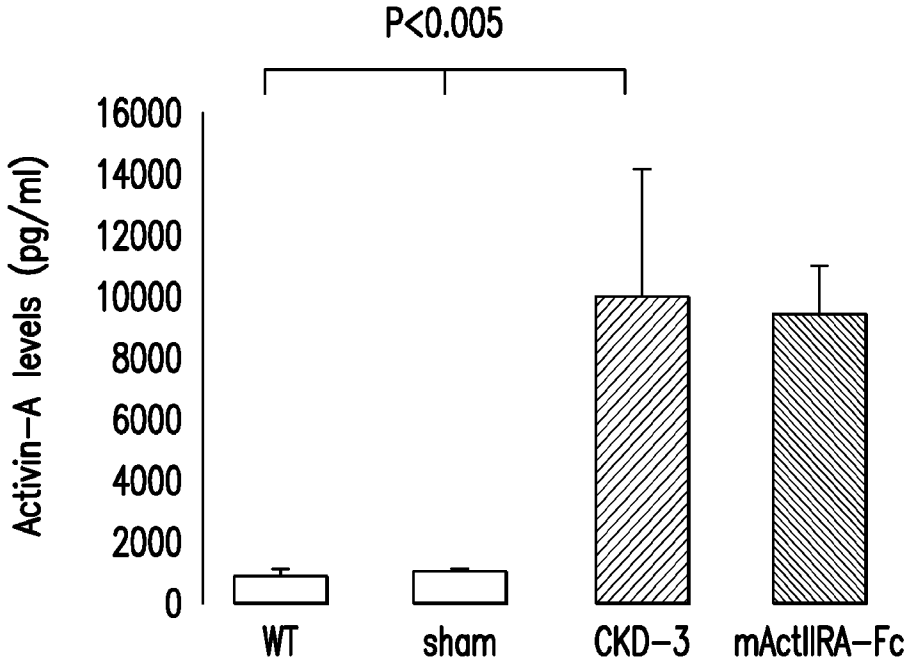

P<0.005
Activin-A levels (pg/ml)
16000
14000
12000
10000
8000
6000
4000
2000
0
WT
sham
CKD-3
mActIIRA-Fc

FIG. 1A

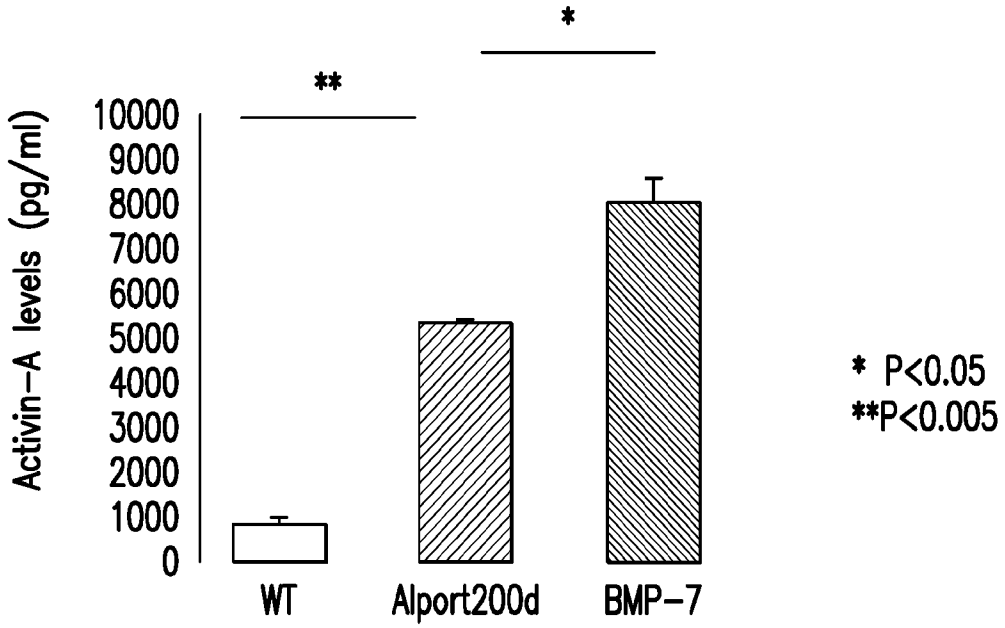

*
**
Activin-A levels (pg/ml)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
WT
Alport200d
BMP-7
* P<0.05
**P<0.005

FIG. 1B

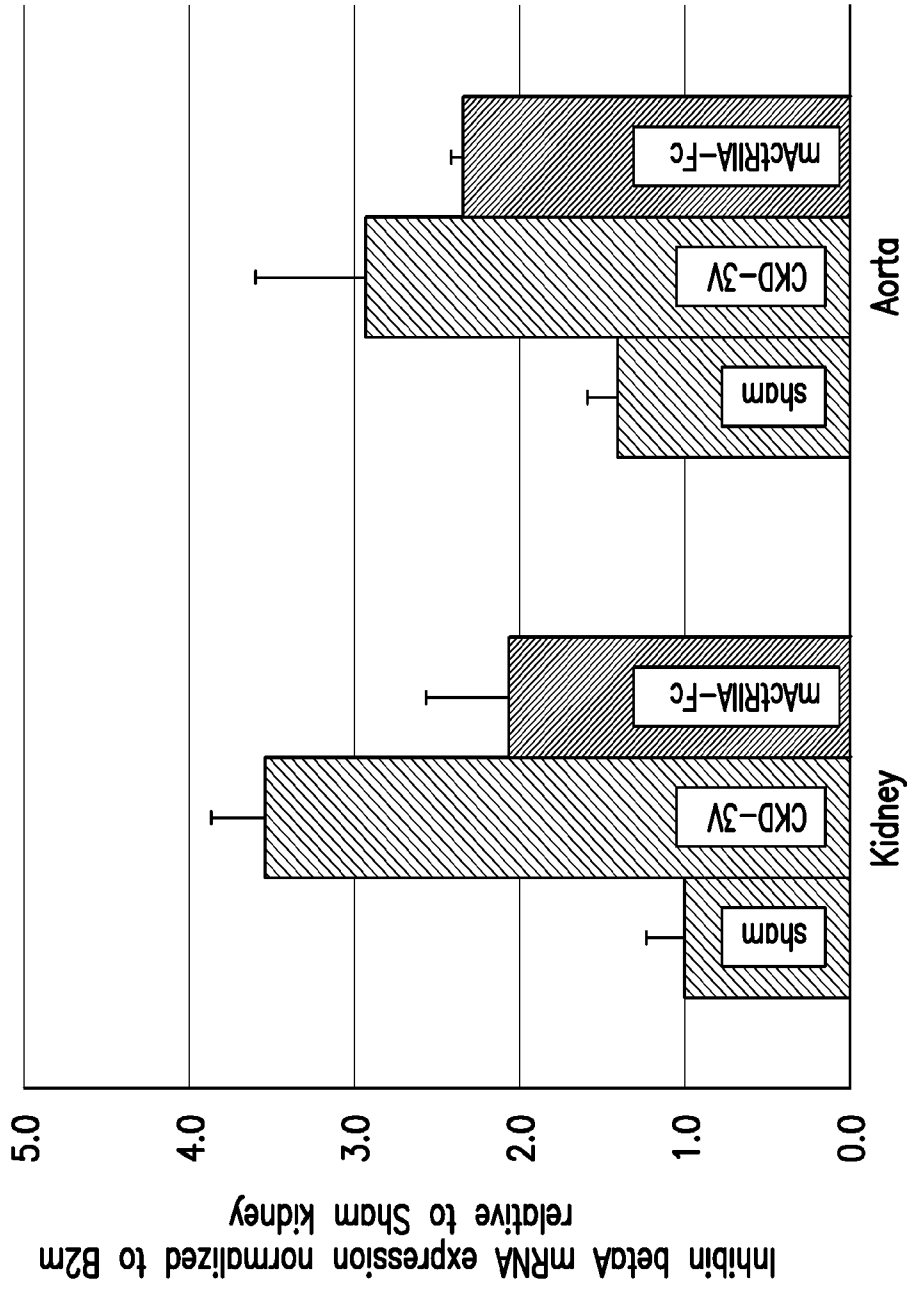

Inhibin betaA mRNA expression normalized to B2m
relative to Sham kidney
5.0
4.0
3.0
2.0
1.0
0.0
Kidney
Aorta
sham
CKD-3V
mActRIIA-Fc
sham
CKD-3V
mActRIIA-Fc

FIG. 2

FIG. 3B

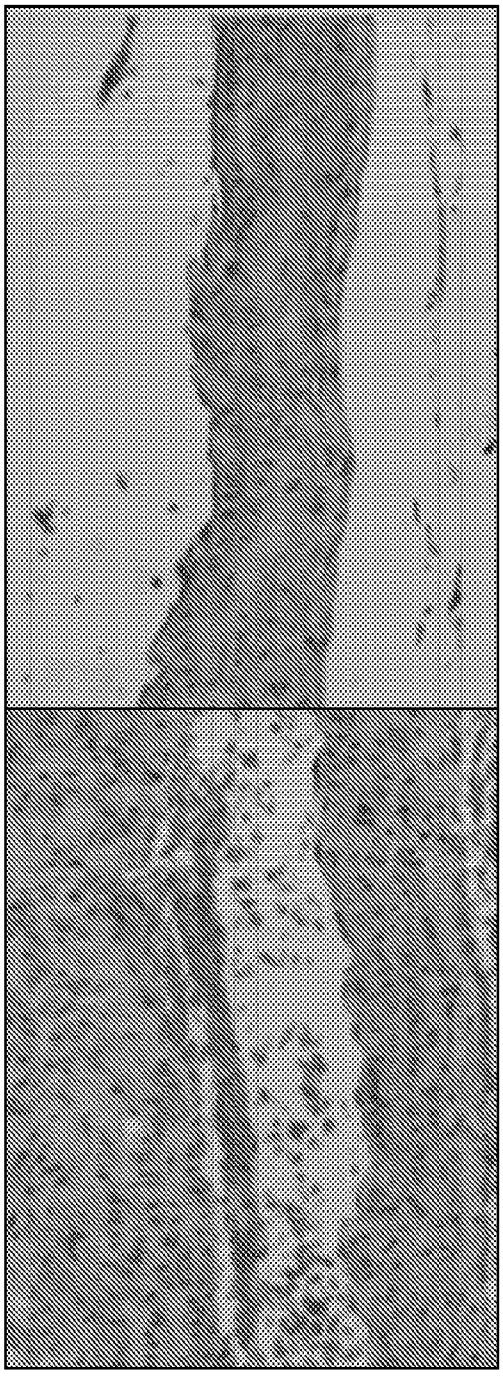


FIG. 3D



FIG. 3A

FIG. 3C

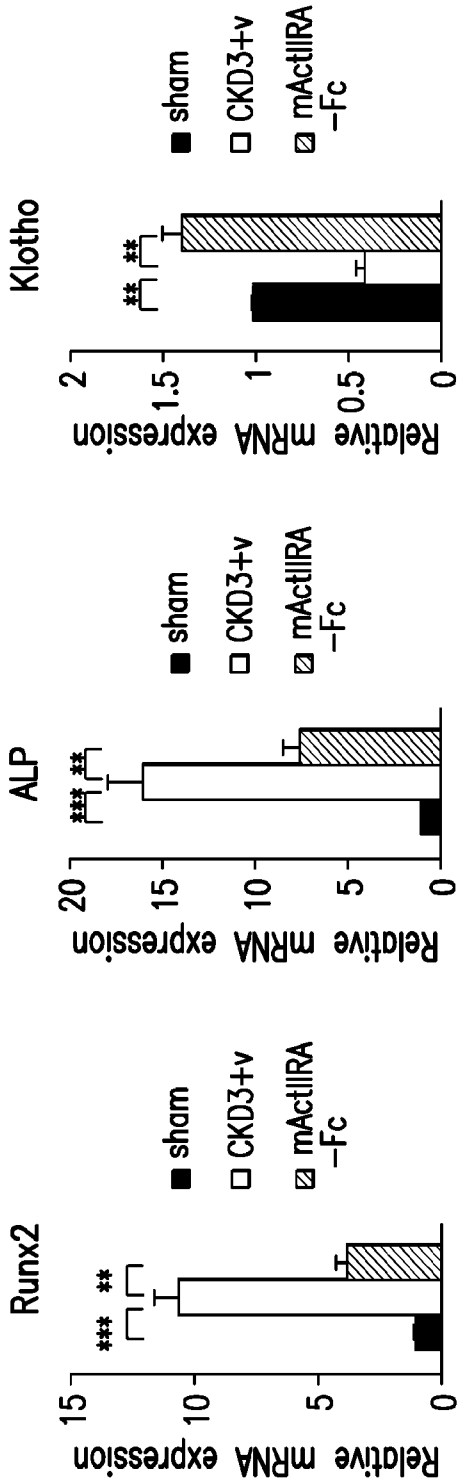


FIG. 4A

FIG. 4B

FIG. 4C

*p<0.05
**p<0.01
***p<0.005

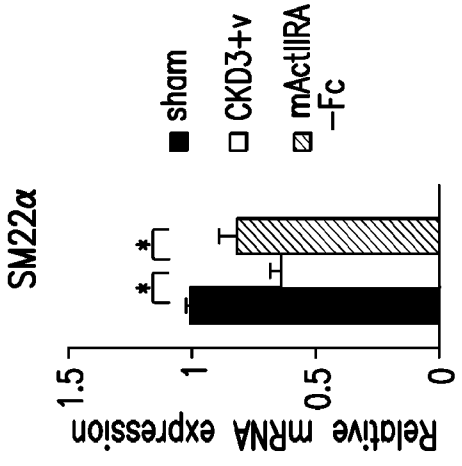


FIG. 4E

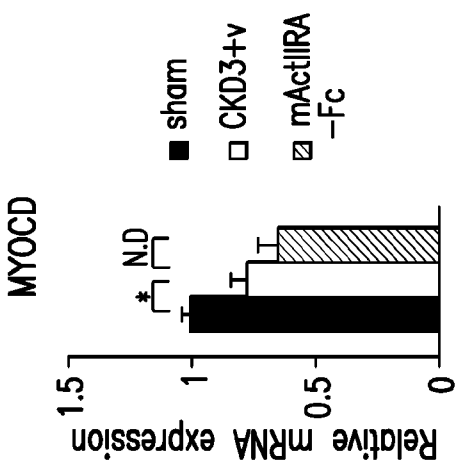


FIG. 4D

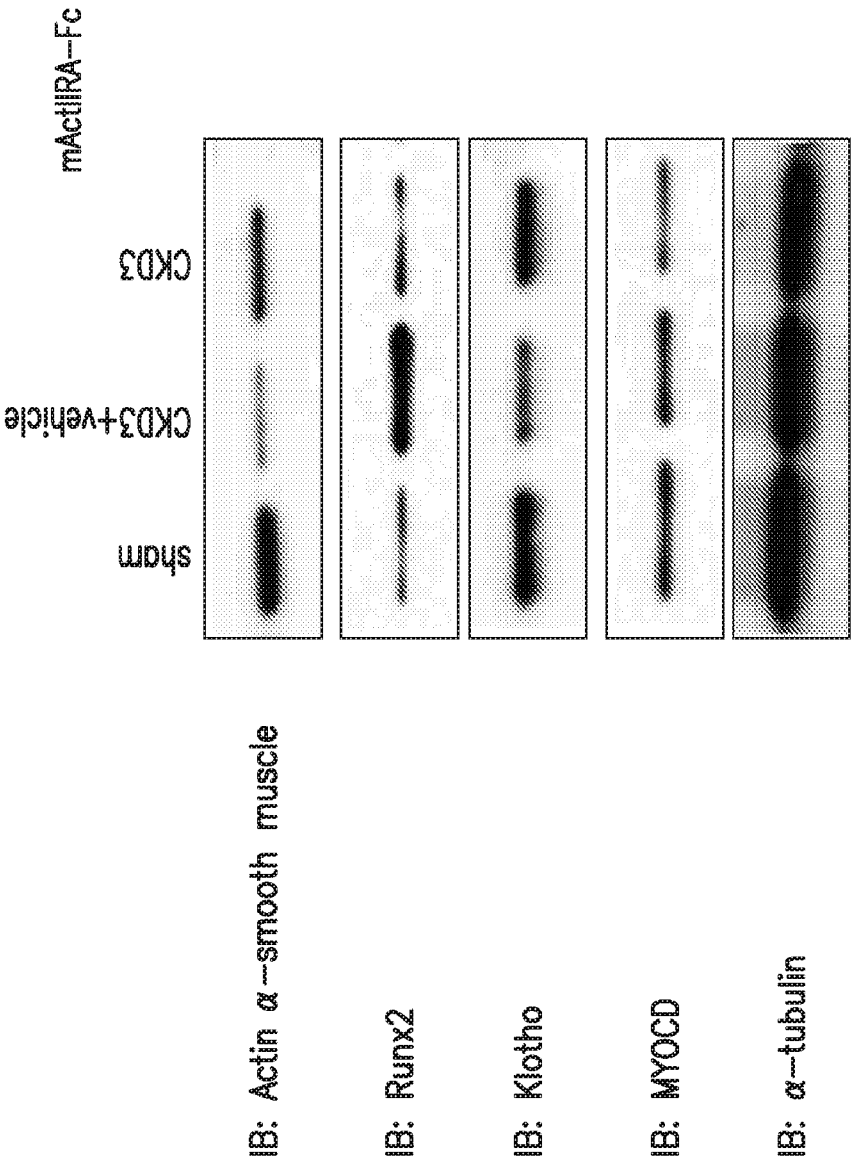
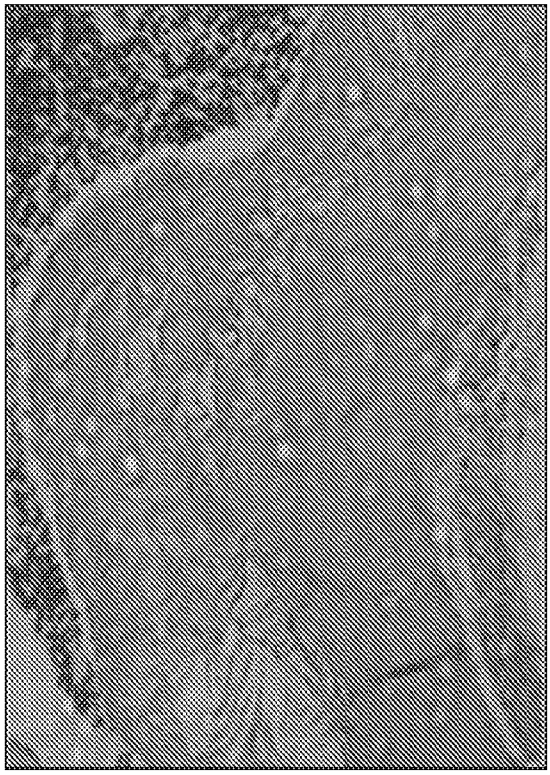

mActIIRA-Fc
sham
CKD3+vehicle
CKD3
IB: Actin α-smooth muscle
IB: Runx2
IB: Klotho
IB: MYOCD
IB: α-tubulin

FIG. 4F



CKD-3 mActIIRA-Fc Rx

FIG. 5B



CKD-3 veh. Rx

FIG. 5A

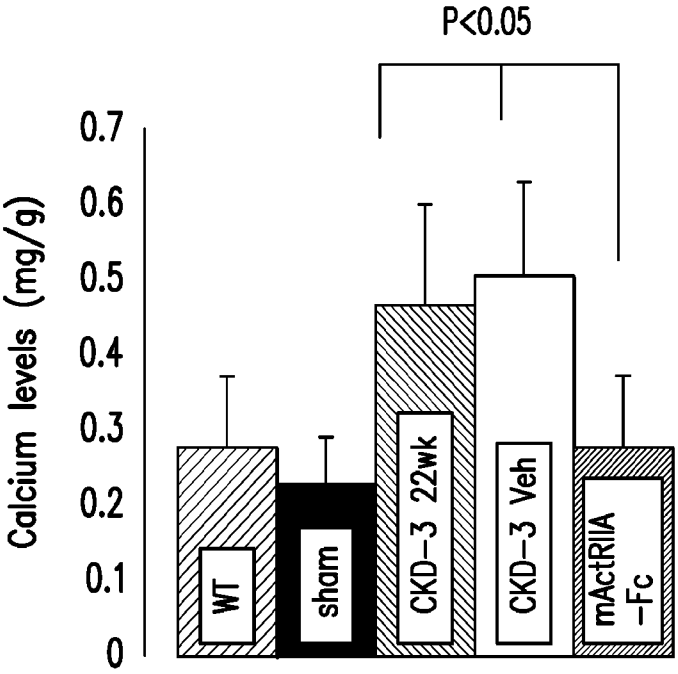

P<0.05
Calcium levels (mg/g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WT
sham
CKD-3 22wk
CKD-3 Veh
mActRIIA -Fc

FIG. 6A

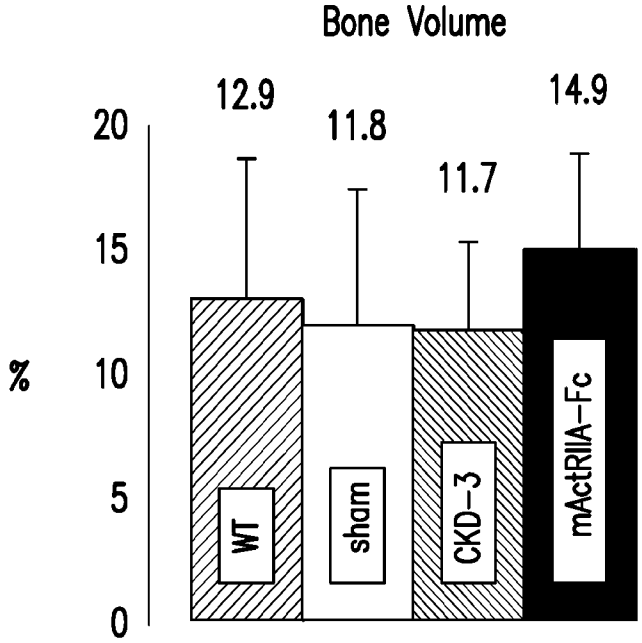
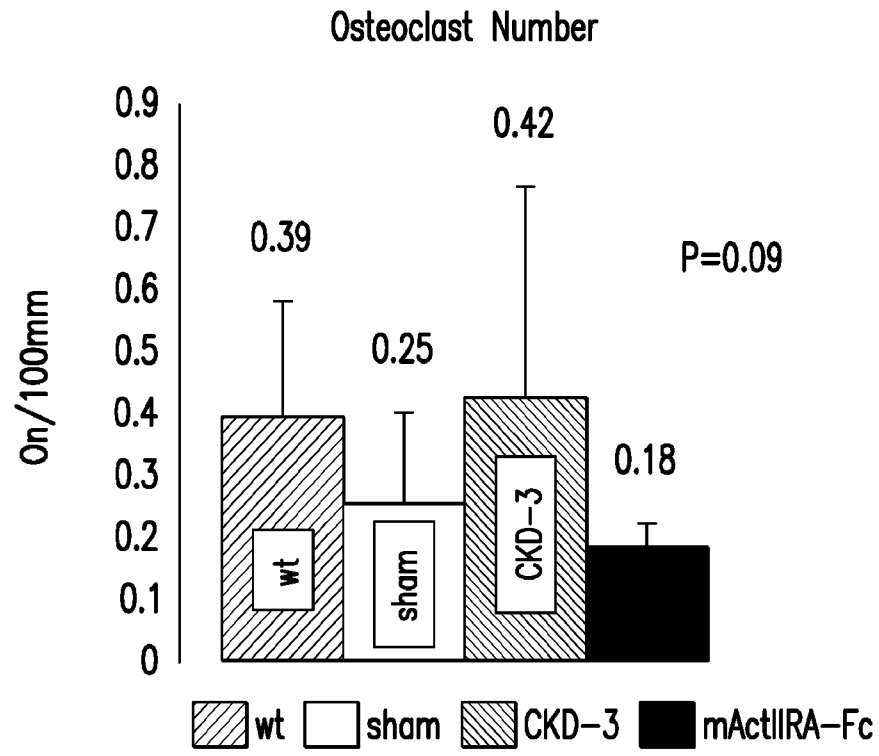

Bone Volume
12.9
11.8
11.7
14.9
20
15
10
5
0
%
WT
sham
CKD-3
mActRIIA-Fc

FIG. 6B


Osteoclast Number
On/100mm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.39
0.25
0.42
0.18
P=0.09
wt
sham
CKD-3
wt
sham
CKD-3
mActIIRA-Fc

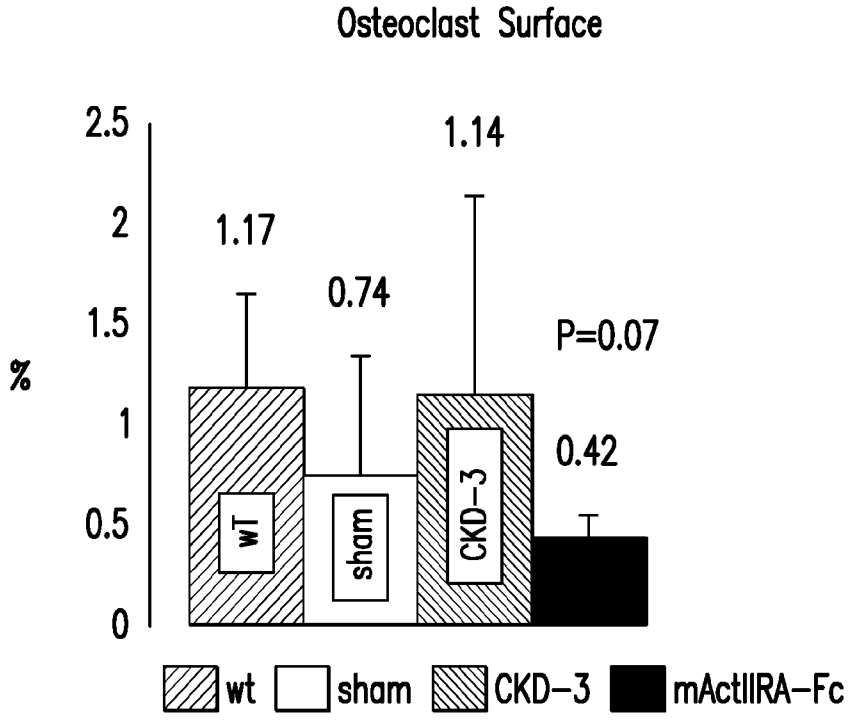

Osteoclast Surface
%
2.5
2
1.5
1
0.5
0
1.17
0.74
1.14
P=0.07
0.42
wT
sham
CKD-3
wt
sham
CKD-3
mActIIRA-Fc

FIG. 6D

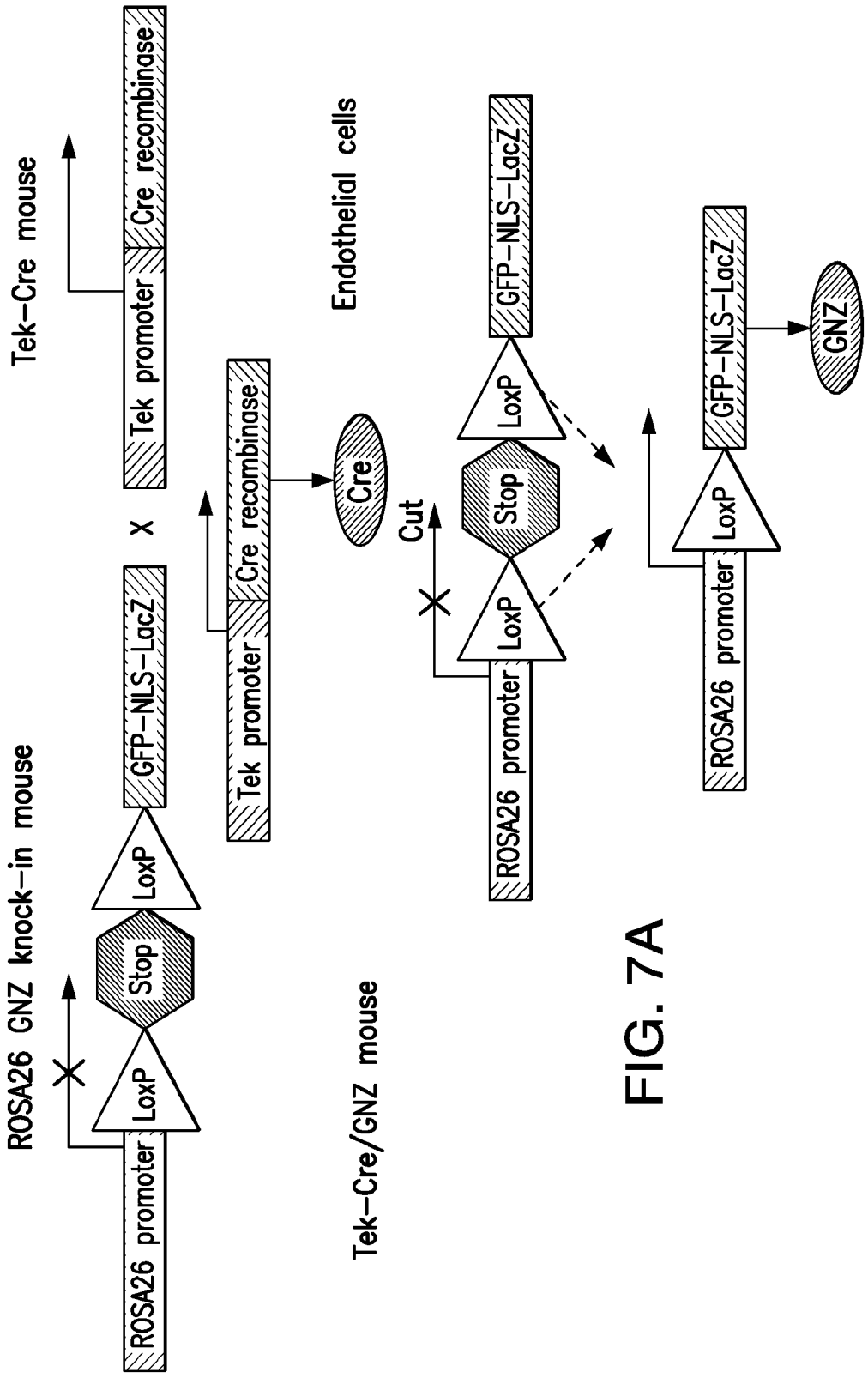

Tek-Cre mouse
Tek promoter
Cre recombinase
X
ROSA26 GNZ knock-in mouse
ROSA26 promoter
LoxP
Stop
LoxP
GFP-NLS-LacZ
Tek-Cre/GNZ mouse
Endothelial cells
Tek promoter
Cre recombinase
Cre
Cut
ROSA26 promoter
LoxP
Stop
LoxP
GFP-NLS-LacZ
ROSA26 promoter
LoxP
GFP-NLS-LacZ
GNZ

FIG. 7A

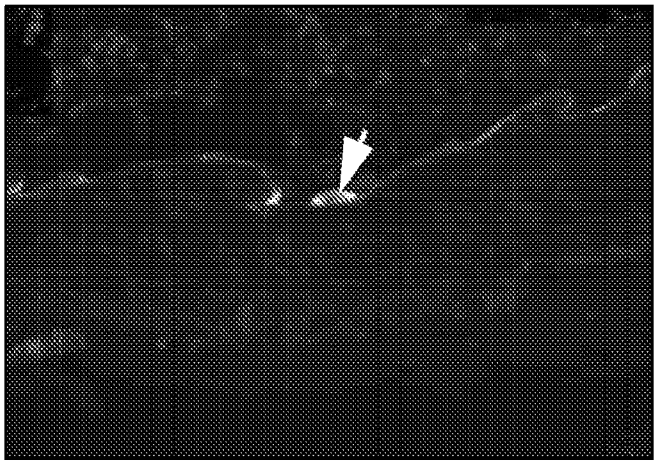


FIG. 7B

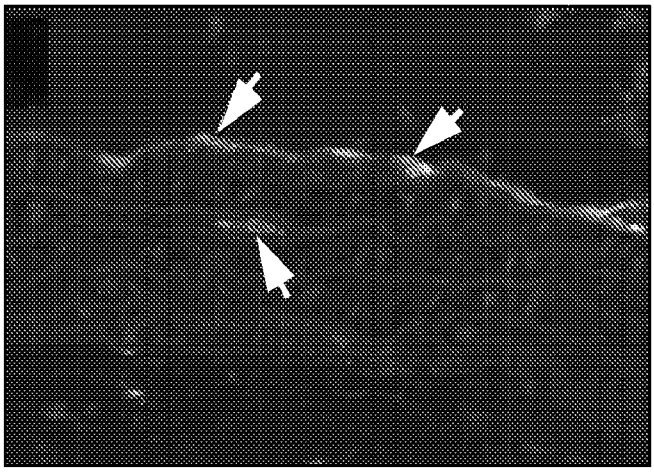


FIG. 7C

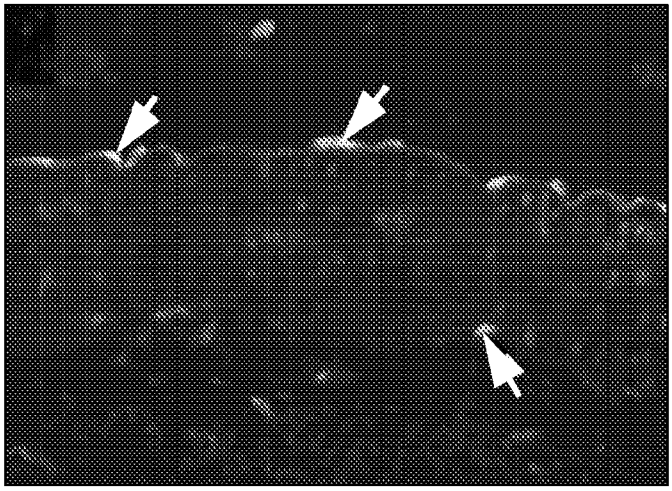


FIG. 7D

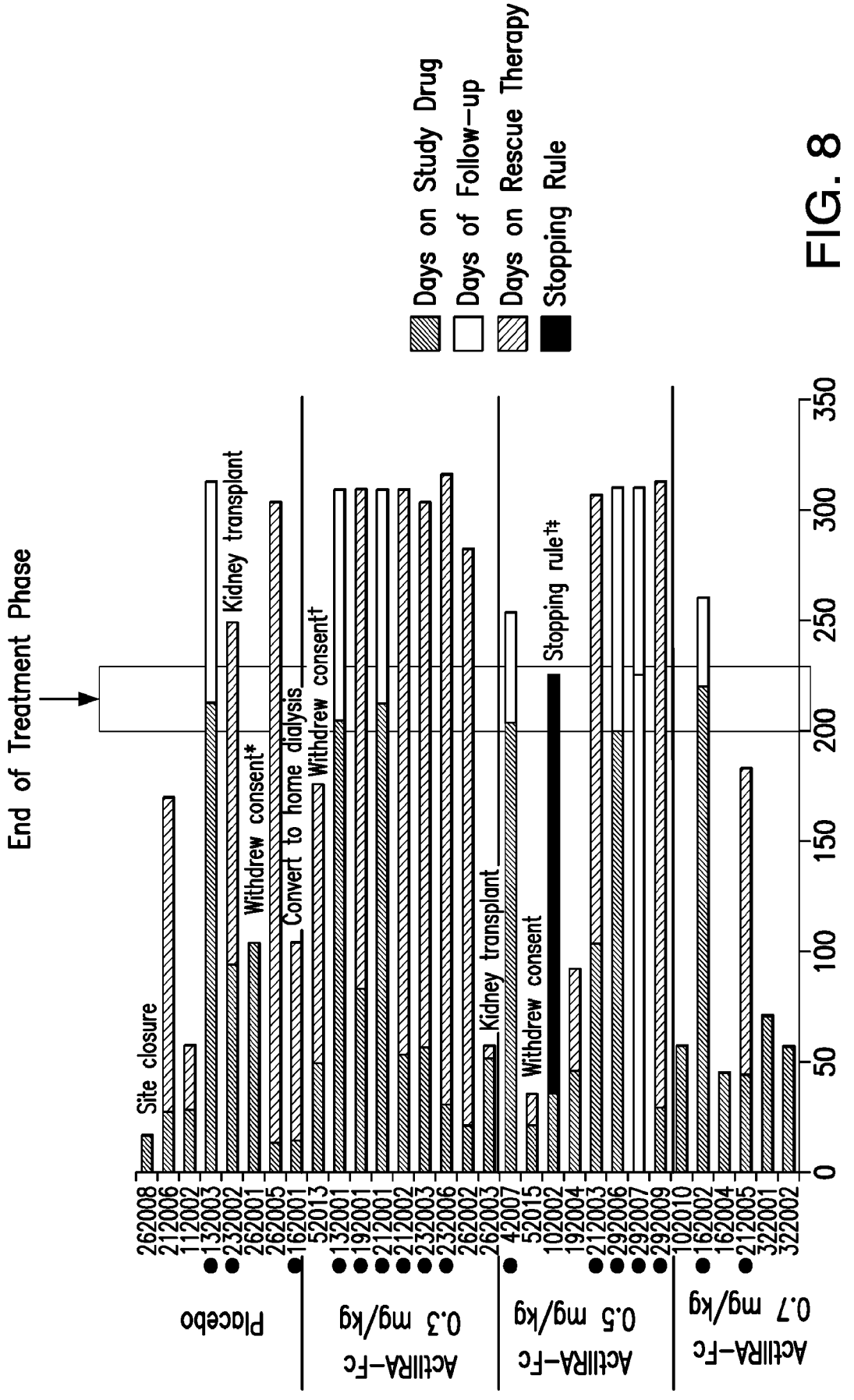

Days on Study Drug
Days of Follow-up
Days on Rescue Therapy
Stopping Rule
End of Treatment Phase
Site closure
Kidney transplant
Withdrew consent*
Convert to home dialysis
Withdrew consent†
Kidney transplant
Withdrew consent
Stopping rule‡
Placebo
ActIIRA-Fc 0.3 mg/kg
ActIIRA-Fc 0.5 mg/kg
ActIIRA-Fc 0.7 mg/kg
262008
212006
112002
132003
232002
262001
262005
162001
52013
132001
192001
212001
212002
232003
232006
262002
262003
42007
52015
102002
192004
212003
292006
292007
292009
102010
162002
162004
212005
322001
322002
0
50
100
150
200
250
300
350
Study Day

FIG. 8

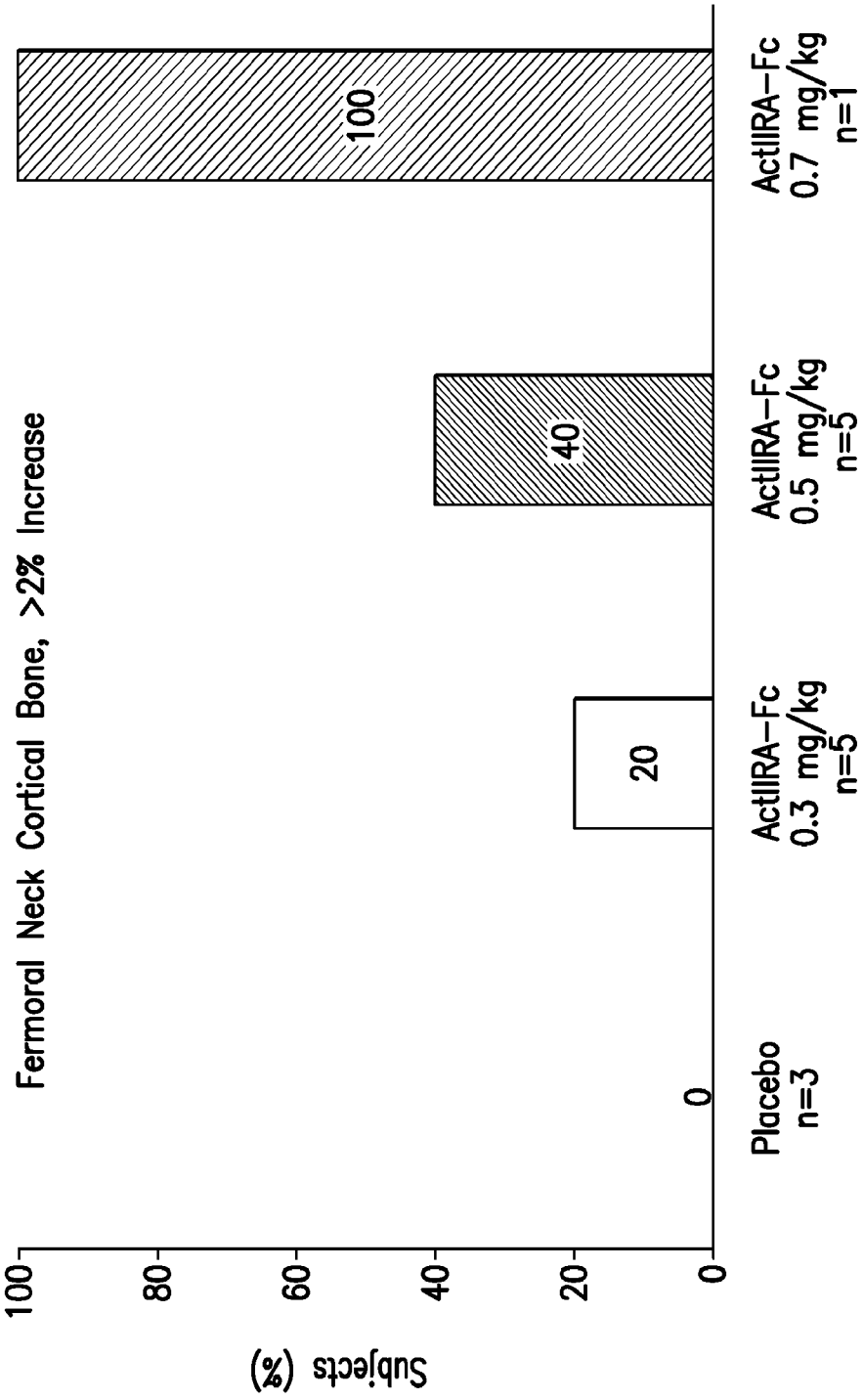

Femoral Neck Cortical Bone, >2% Increase
Subjects (%)
100
80
60
40
20
0
0
20
40
100
Placebo n=3
ActIIRA-Fc 0.3 mg/kg n=5
ActIIRA-Fc 0.5 mg/kg n=5
ActIIRA-Fc 0.7 mg/kg n=1

FIG. 9A

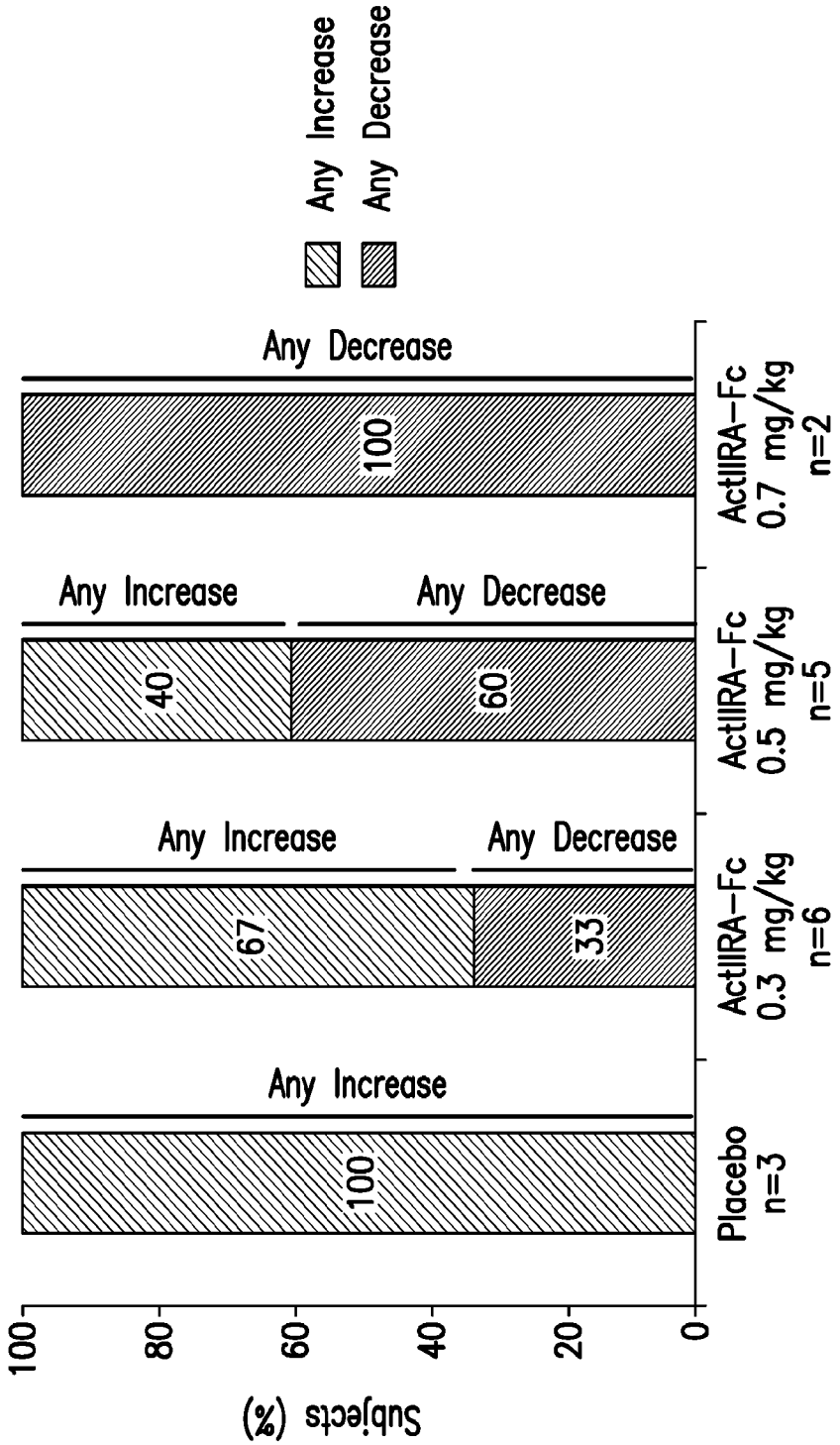

Any Increase
Any Decrease
Any Decrease
100
ActIIRA-Fc
0.7 mg/kg
n=2
Any Increase
Any Decrease
40
60
ActIIRA-Fc
0.5 mg/kg
n=5
Any Increase
Any Decrease
67
33
ActIIRA-Fc
0.3 mg/kg
n=6
Any Increase
100
Placebo
n=3
100
80
60
40
20
0
Subjects (%)

FIG. 9B

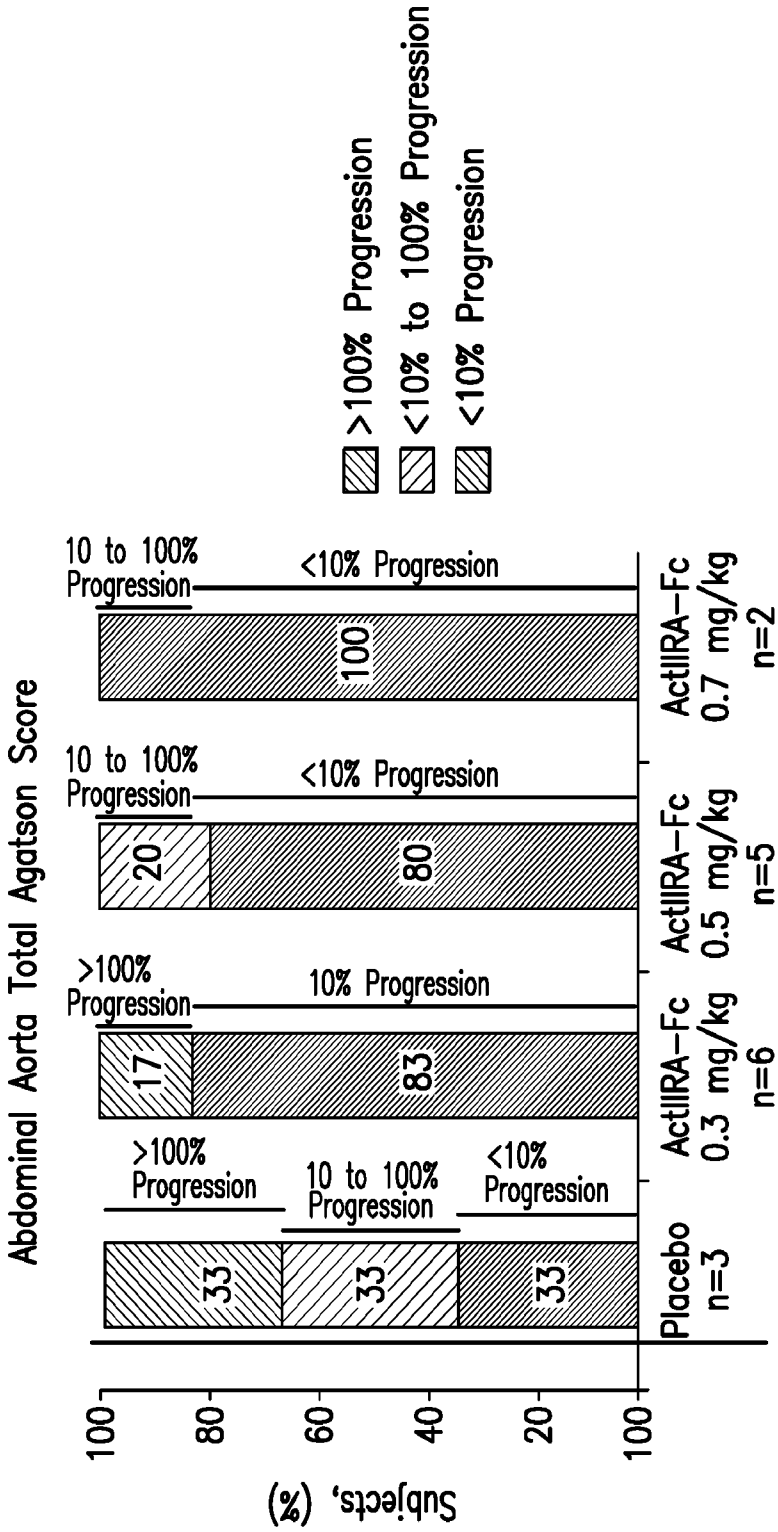

>100% Progression
<10% to 100% Progression
<10% Progression
Abdominal Aorta Total Agatson Score
10 to 100% Progression
<10% Progression
100
ActIIRA-Fc 0.7 mg/kg n=2
10 to 100% Progression
<10% Progression
20
80
ActIIRA-Fc 0.5 mg/kg n=5
>100% Progression
10% Progression
17
83
ActIIRA-Fc 0.3 mg/kg n=6
>100% Progression
10 to 100% Progression
<10% Progression
33
33
33
Placebo n=3
100
80
60
40
20
100
Subjects, (%)

FIG. 10A

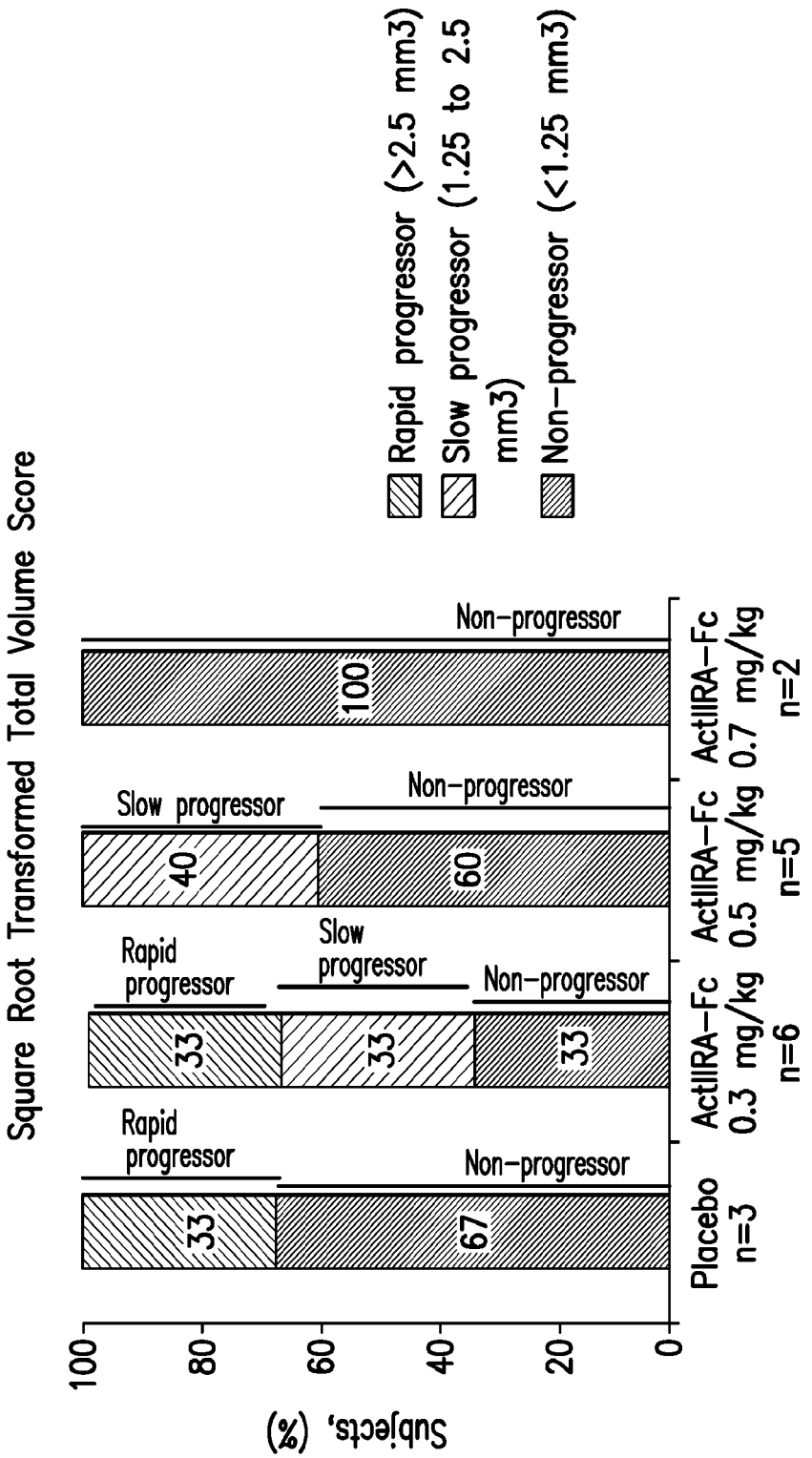
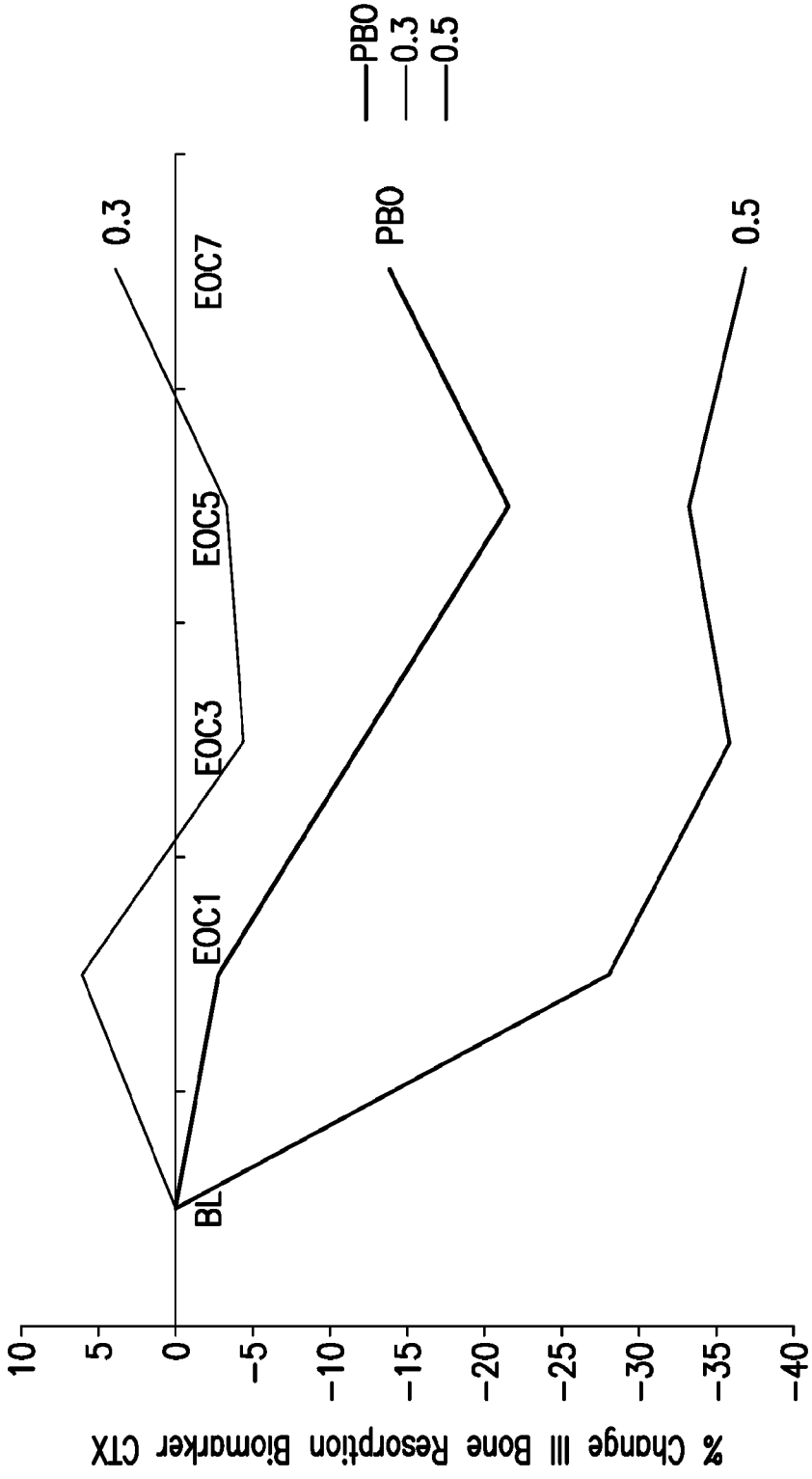

Square Root Transformed Total Volume Score
Rapid progressor (>2.5 mm3)
Slow progressor (1.25 to 2.5 mm3)
Non-progressor (<1.25 mm3)
Subjects, (%)
100
80
60
40
20
0
Placebo n=3
ActIIRA-Fc 0.3 mg/kg n=6
ActIIRA-Fc 0.5 mg/kg n=5
ActIIRA-Fc 0.7 mg/kg n=2
Rapid progressor
Slow progressor
Non-progressor
33
67
40
60
100

FIG. 10B



EOC=end of dose cycle.

FIG. 11

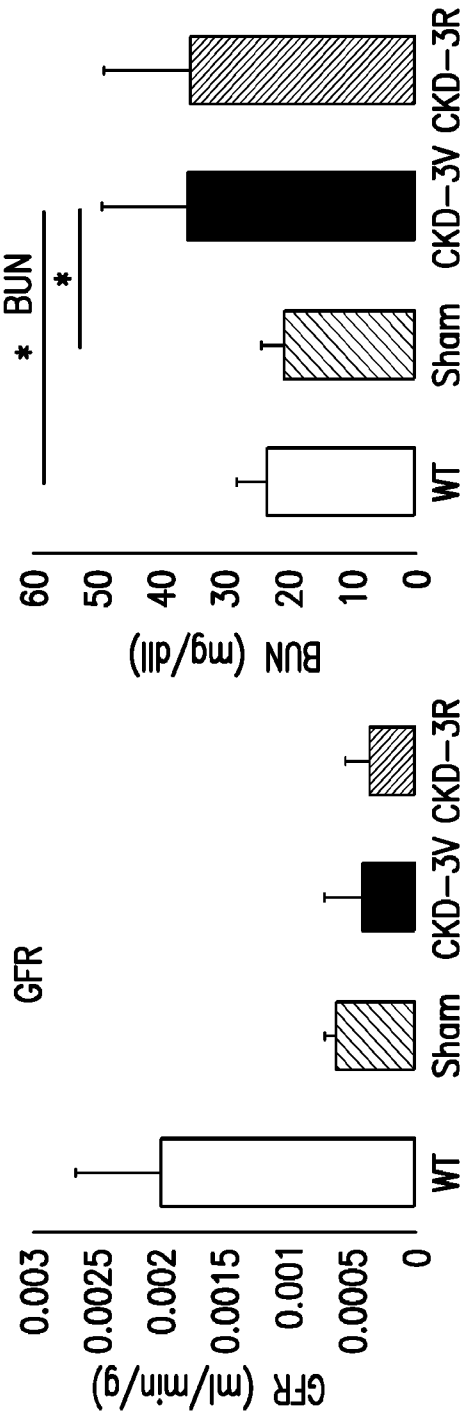


FIG. 12A

FIG. 12B

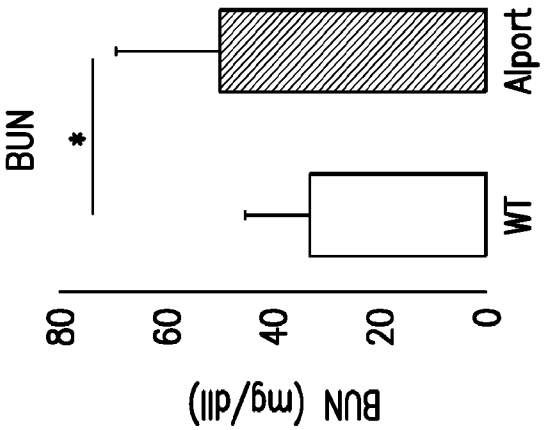


FIG. 12D

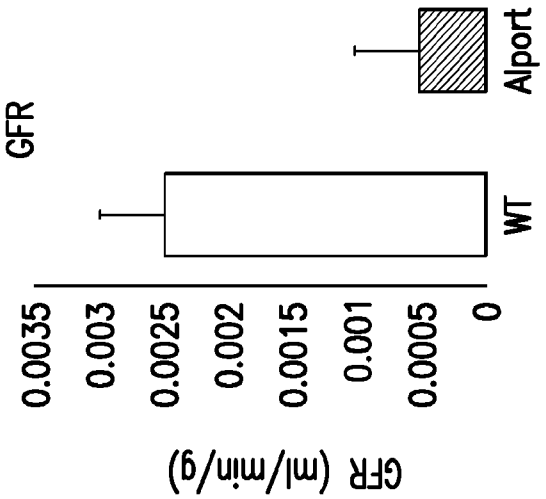


FIG. 12C

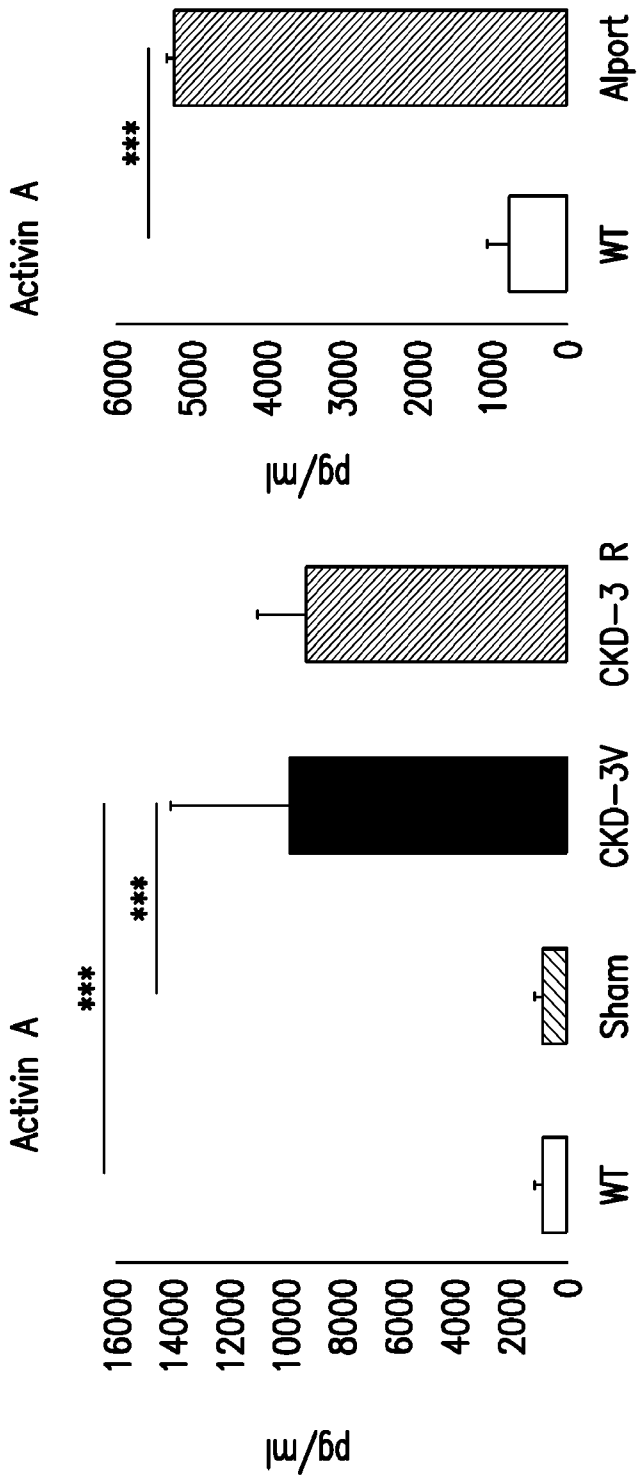


FIG. 13A

FIG. 13B

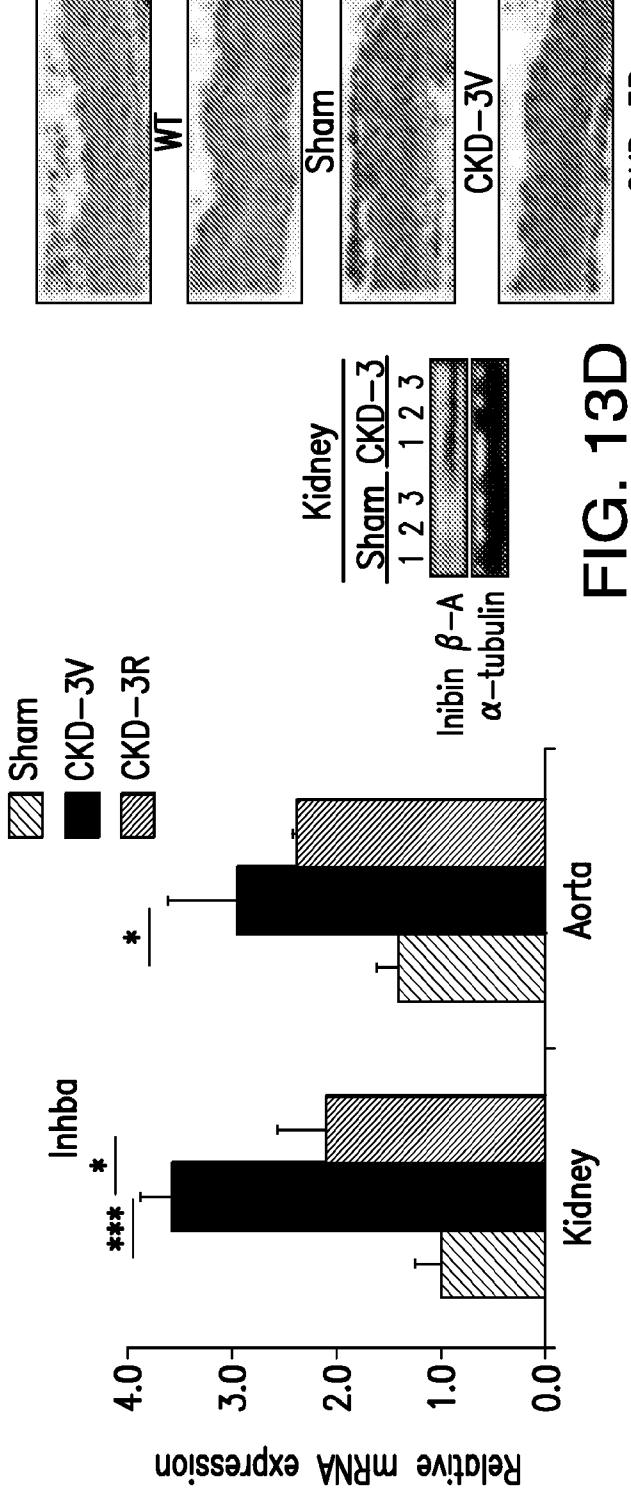


FIG. 13C

FIG. 13D

FIG. 13E

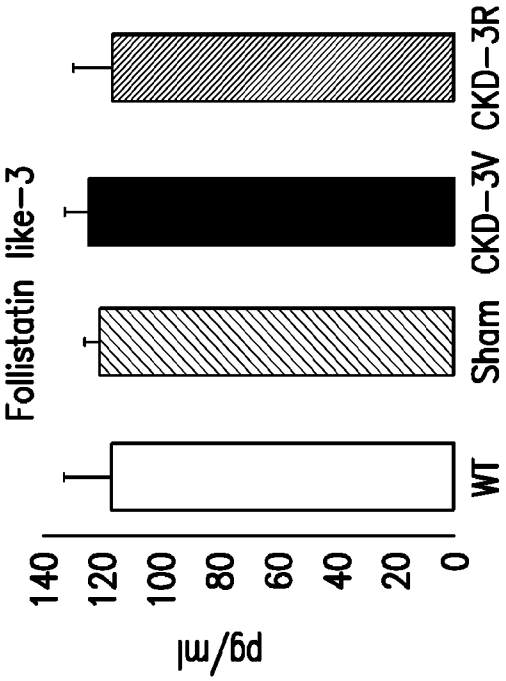


FIG. 13G

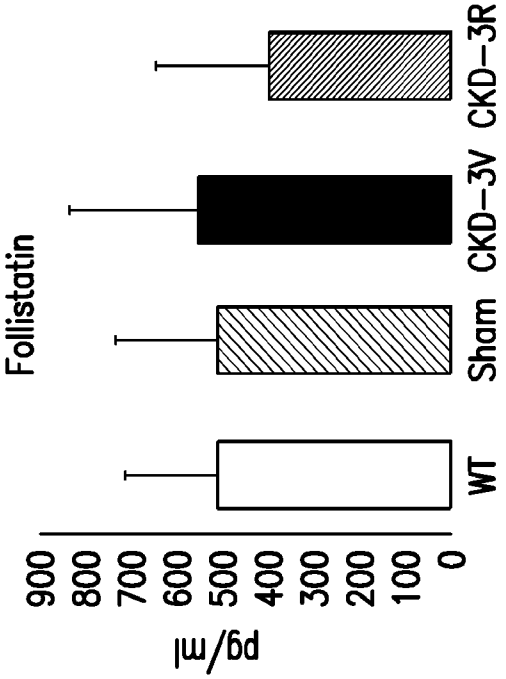


FIG. 13F

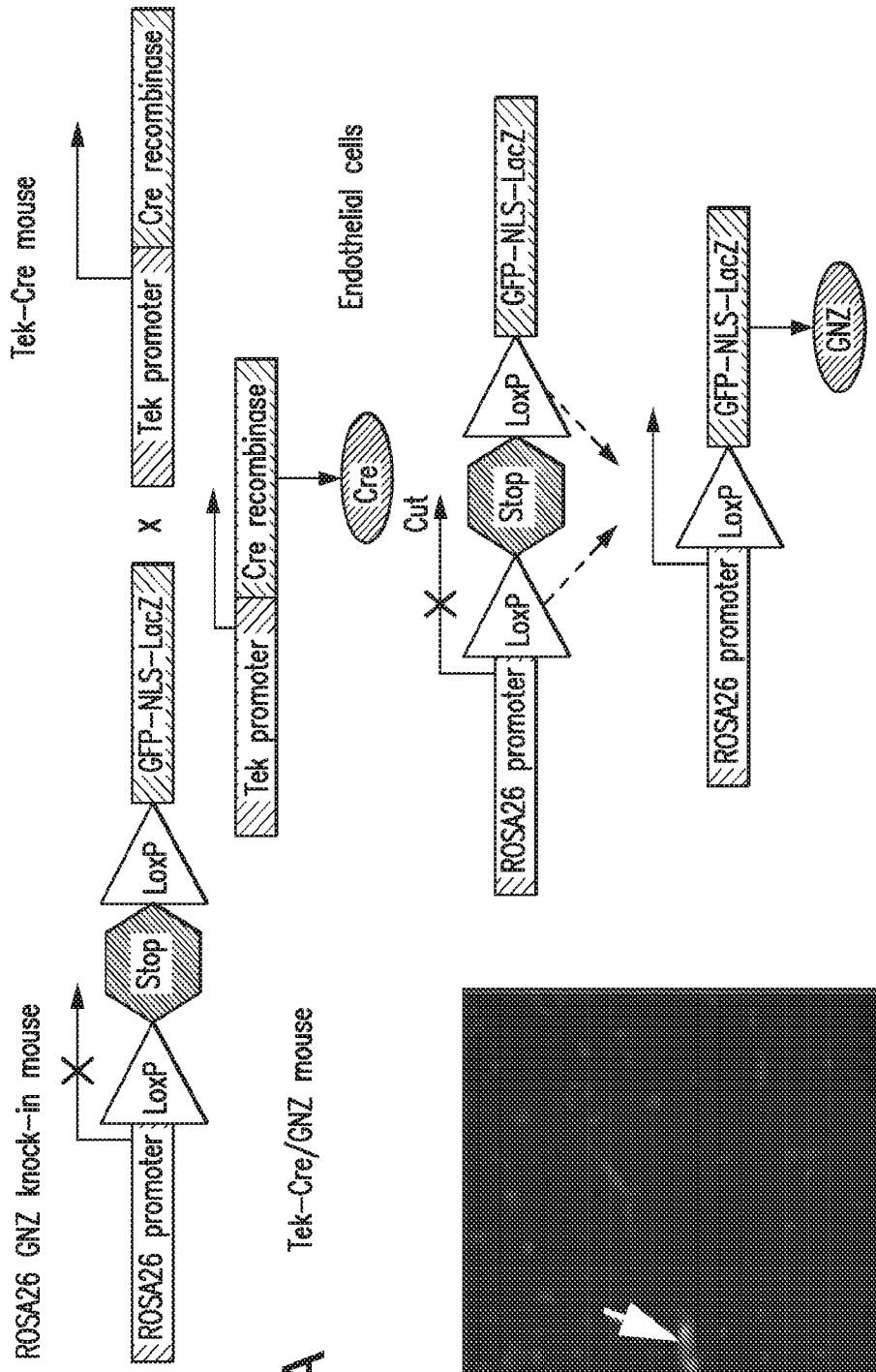


FIG. 14A Tek-Cre/GNZ mouse

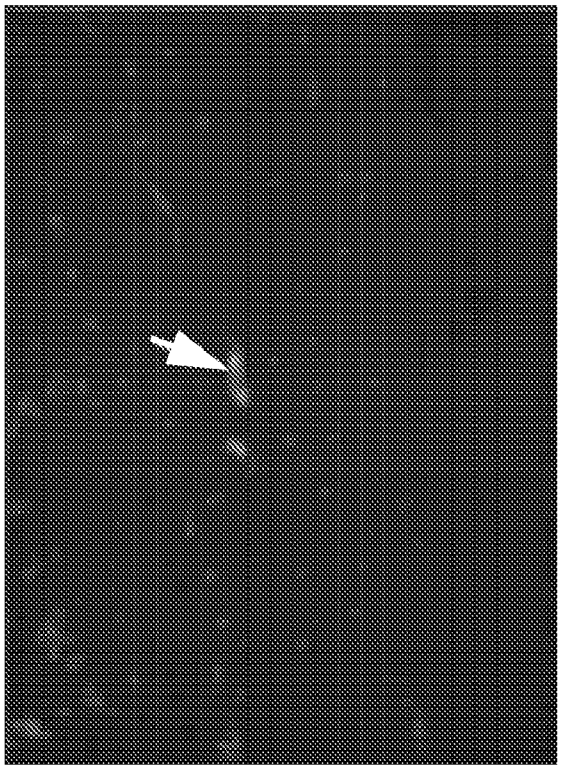


FIG. 14B

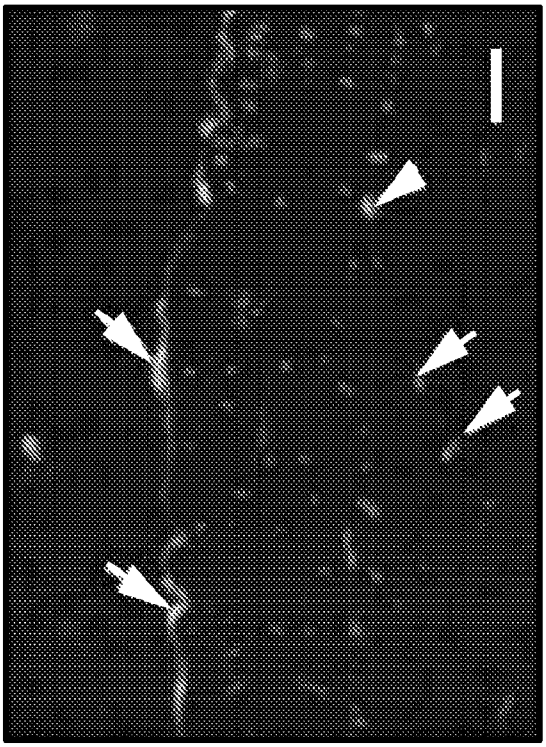


FIG. 14D

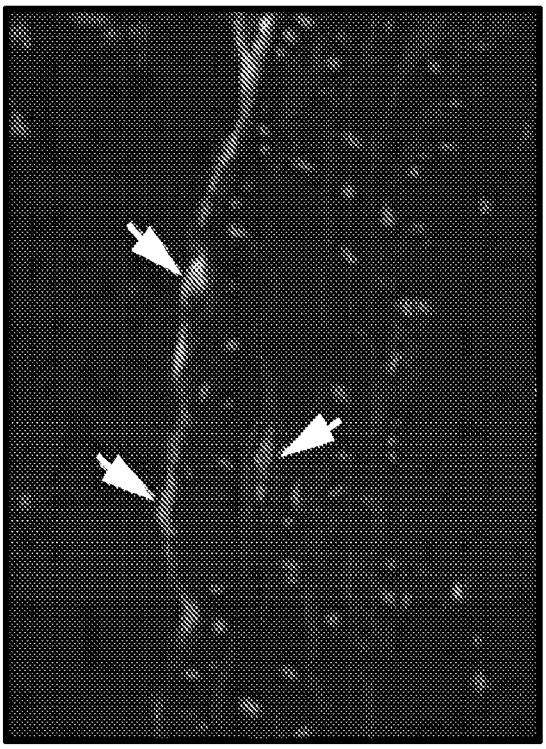


FIG. 14C

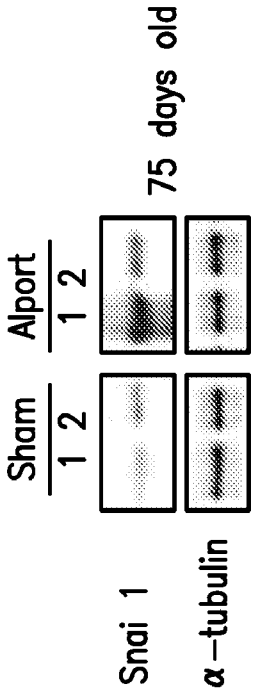


FIG. 14E

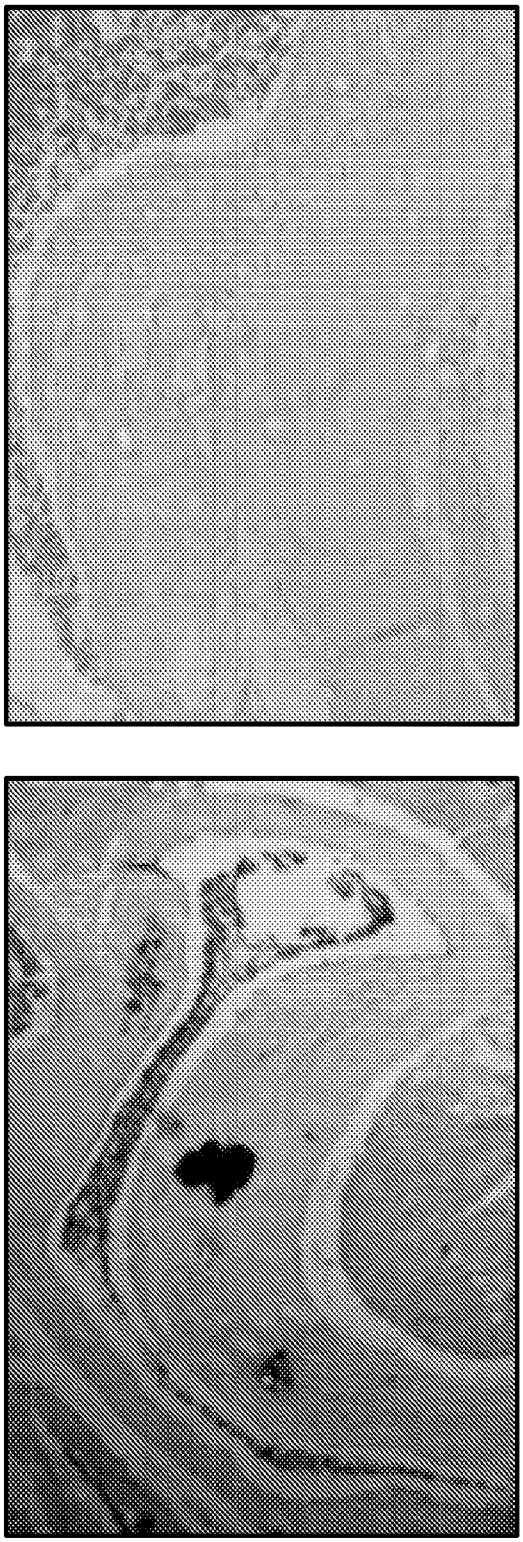


FIG. 15A

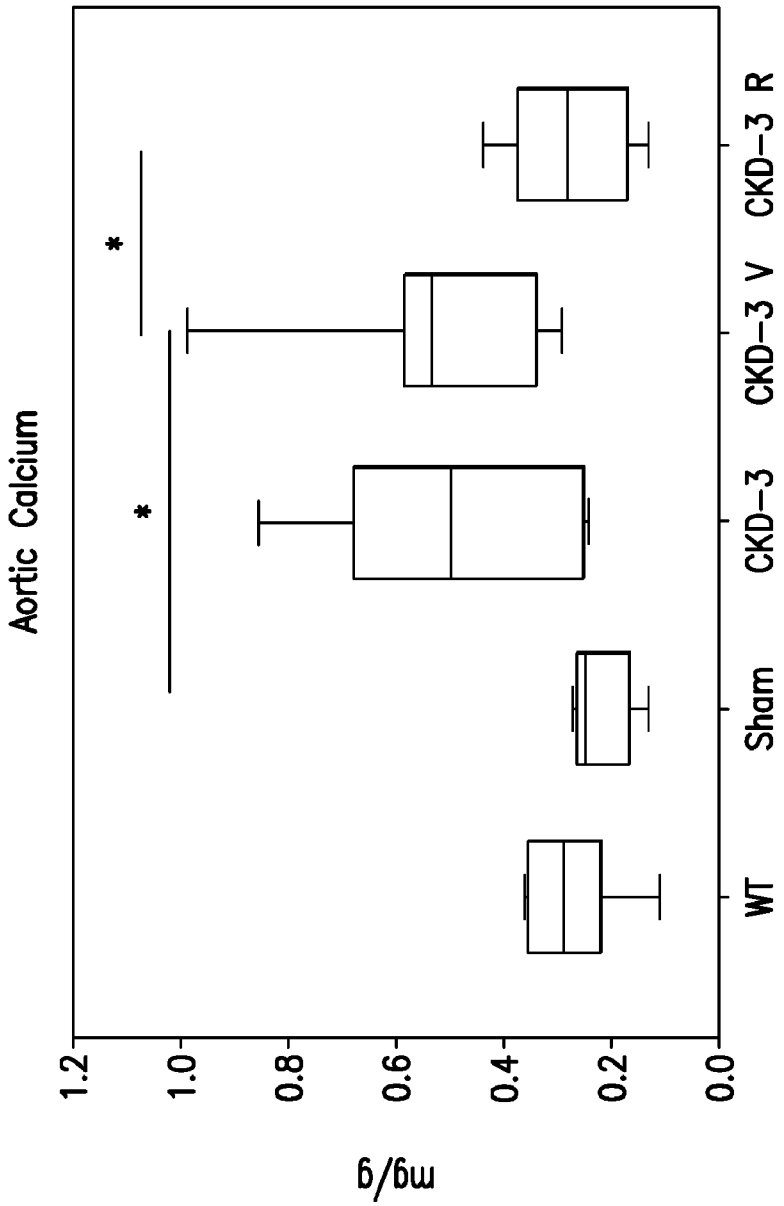

Aortic Calcium
mg/g
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
WT
Sham
CKD-3
CKD-3 V
CKD-3 R

FIG. 15B

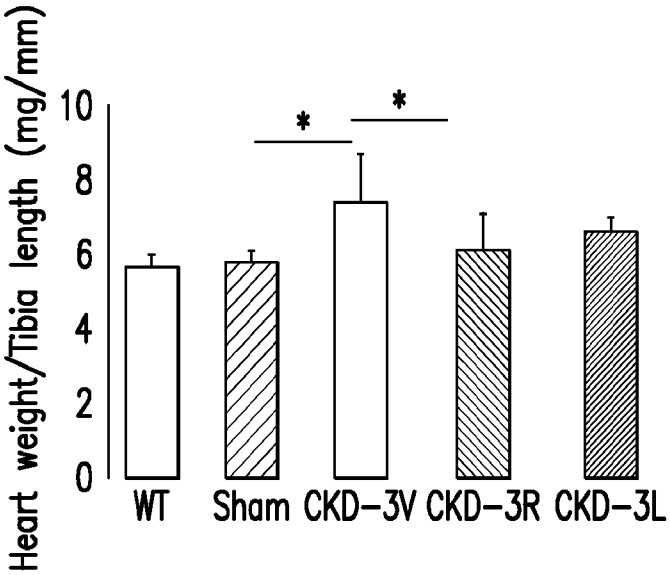


FIG. 16A

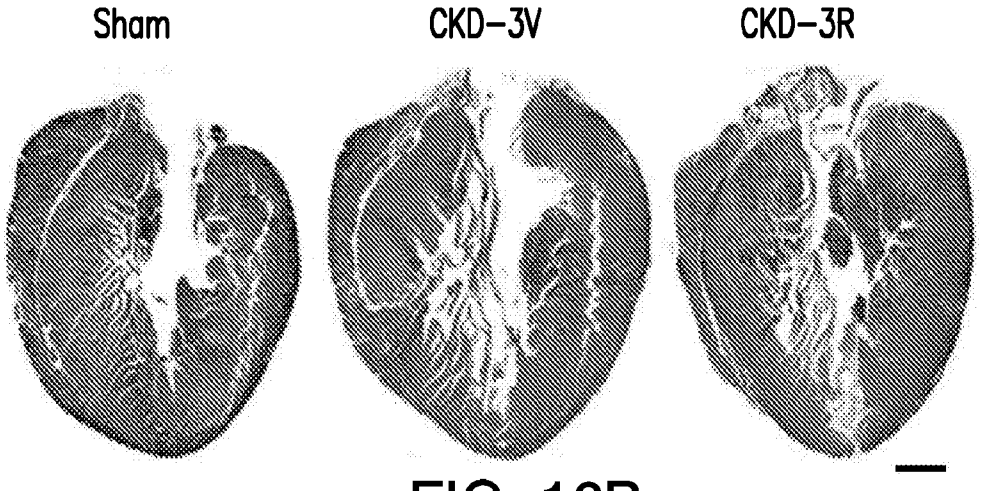


FIG. 16B

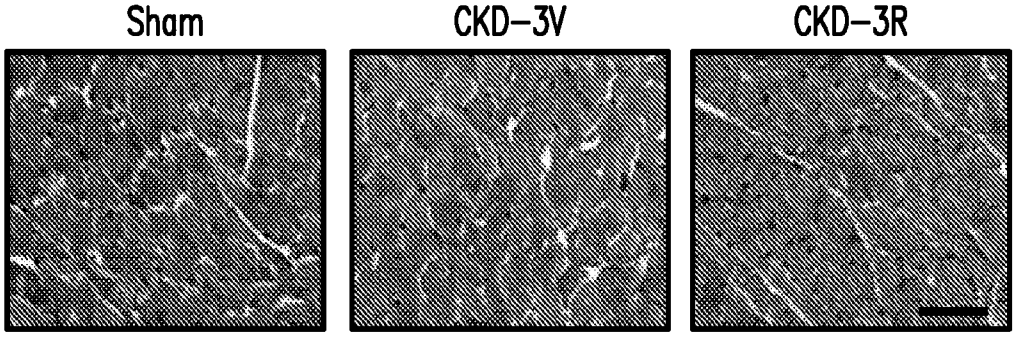


FIG. 16C

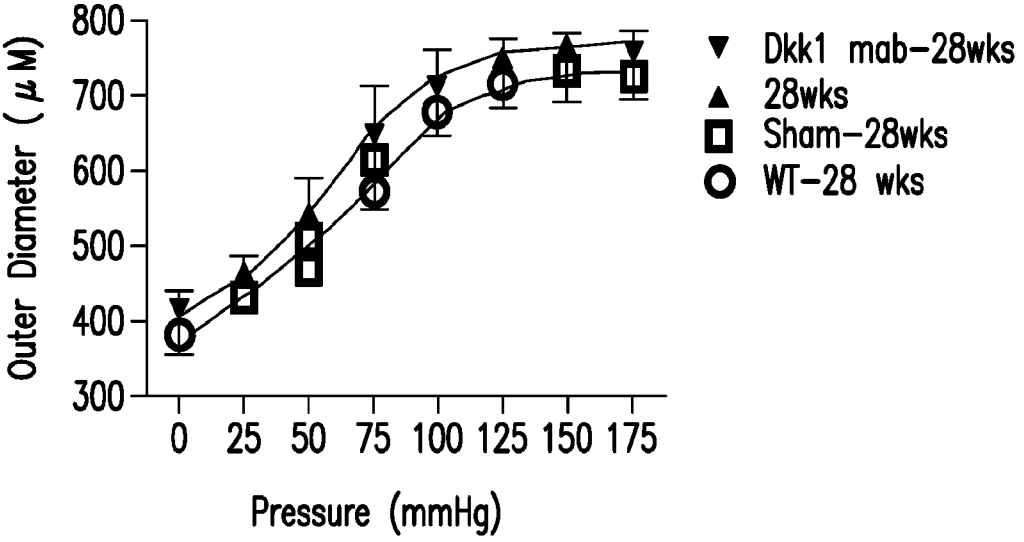


FIG. 17A

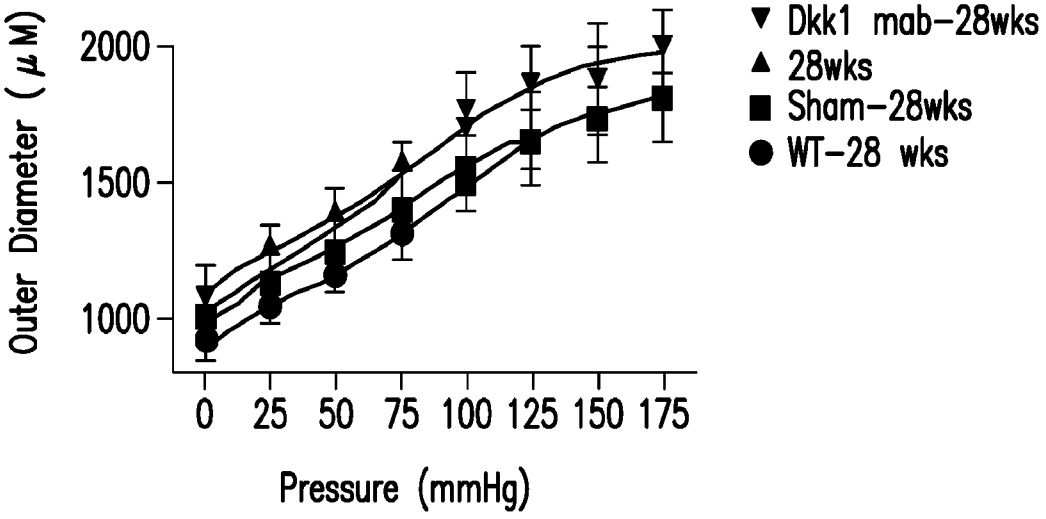


FIG. 17B

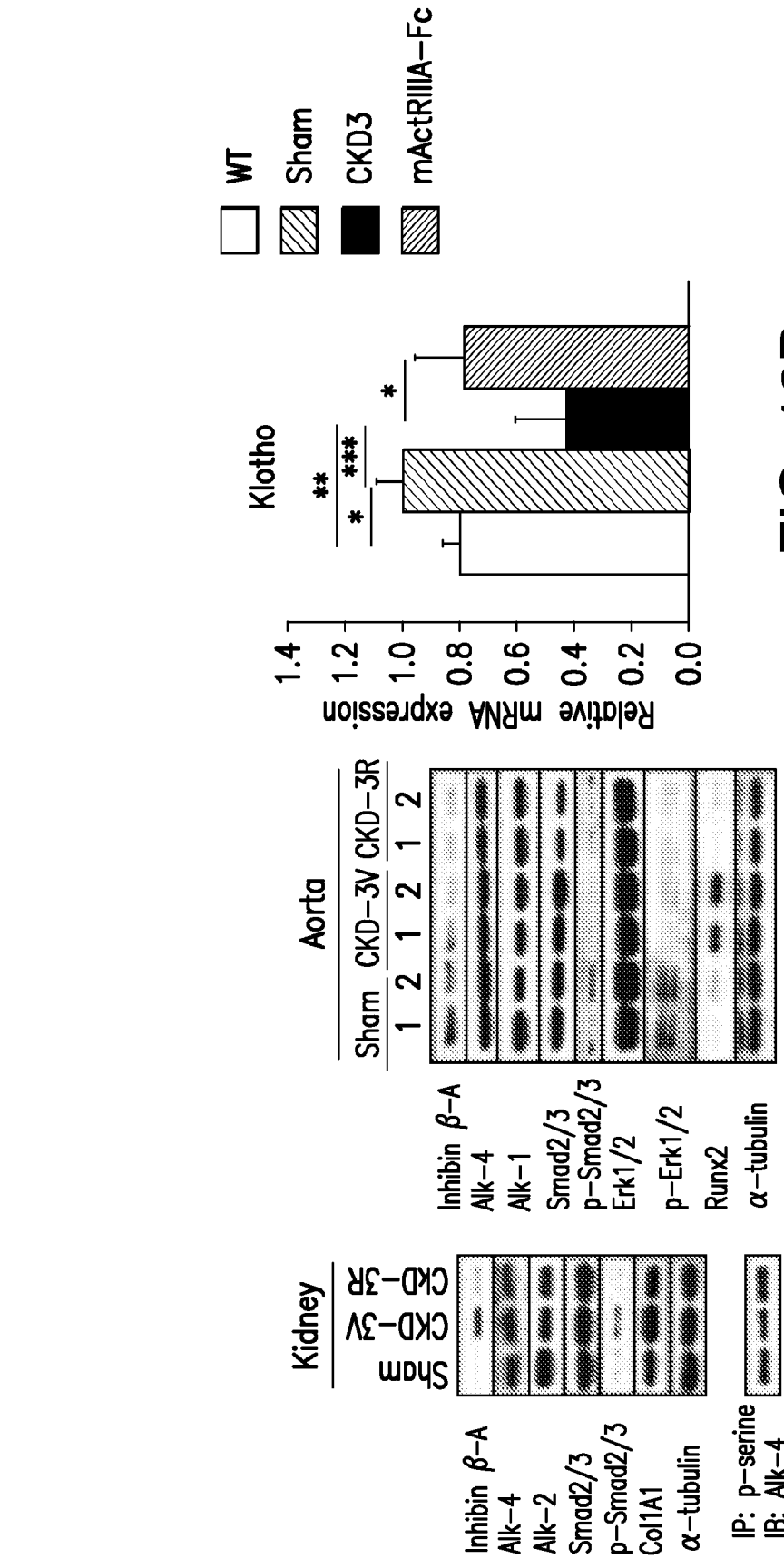


FIG. 18A

FIG. 18B

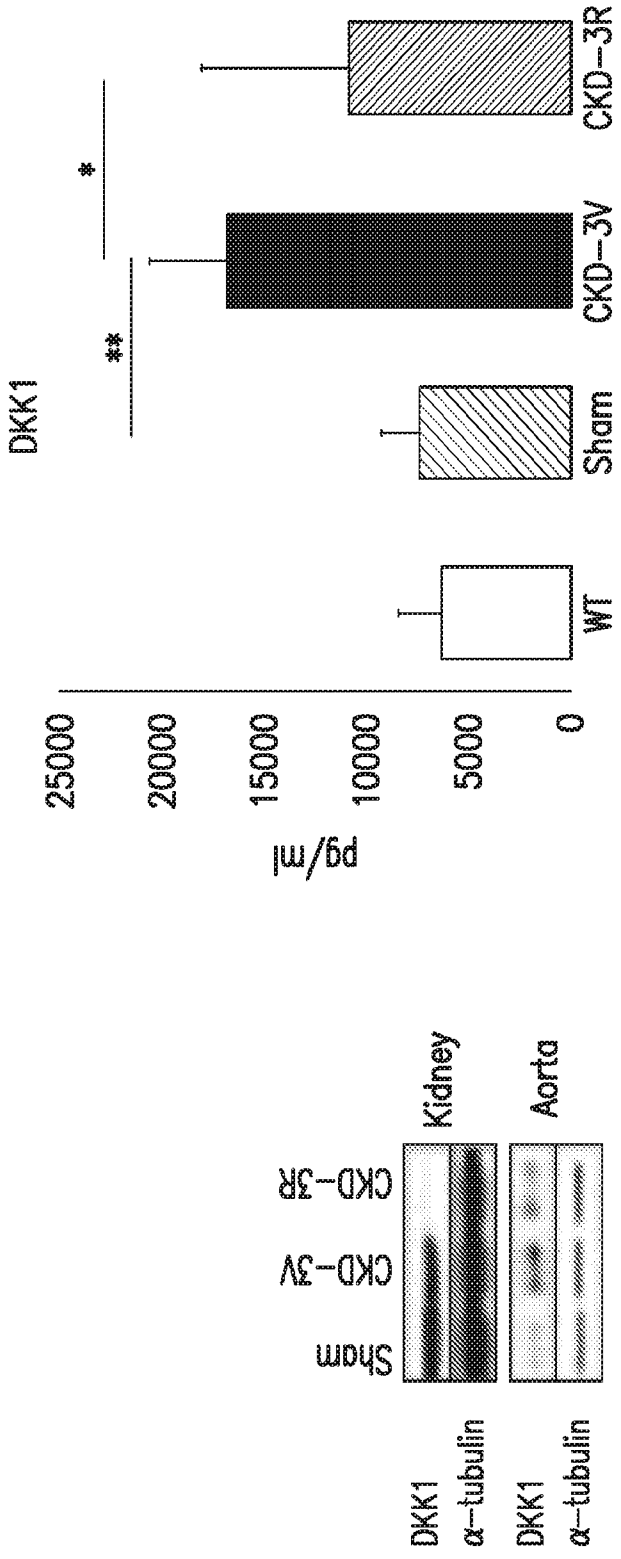

DKK1
pg/ml
25000
20000
15000
10000
5000
0
WT
Sham
CKD-3V
CKD-3R
*
**
Kidney
Aorta
Sham
CKD-3V
CKD-3R
DKK1
α-tubulin
DKK1
α-tubulin

FIG. 18C

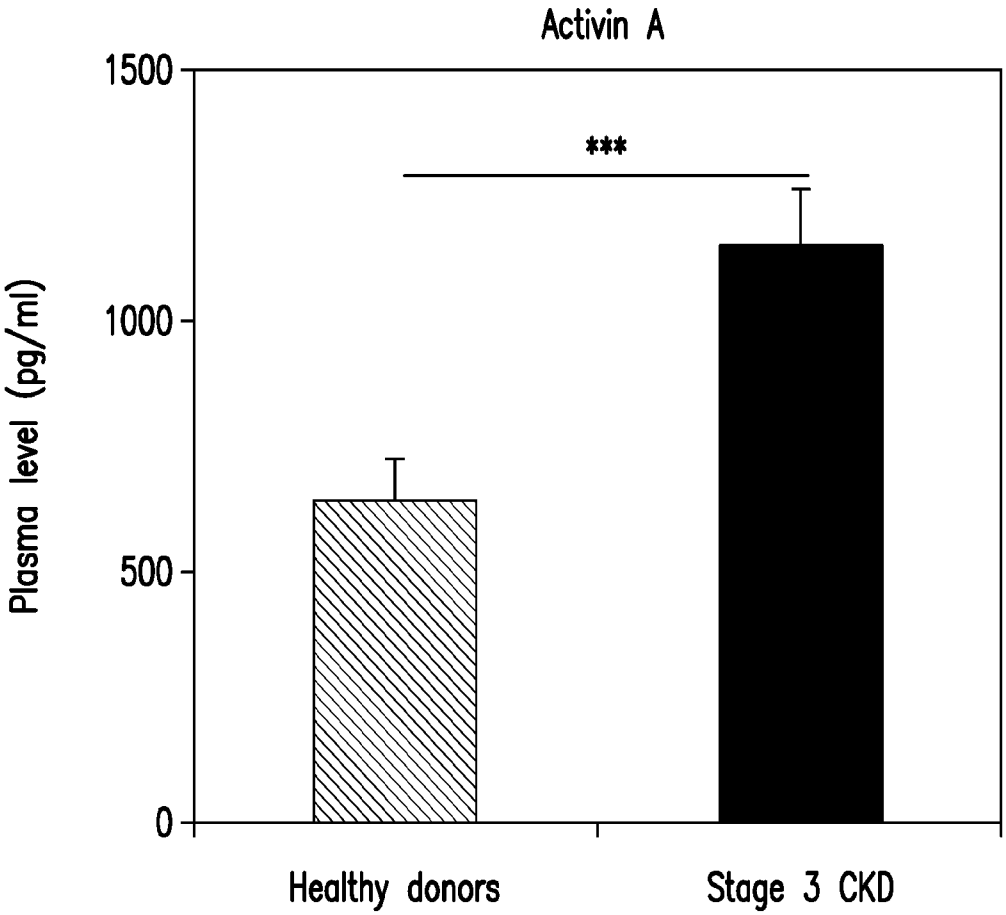

Activin A
1500
1000
500
0
Plasma level (pg/ml)
***
Healthy donors
Stage 3 CKD

FIG. 19

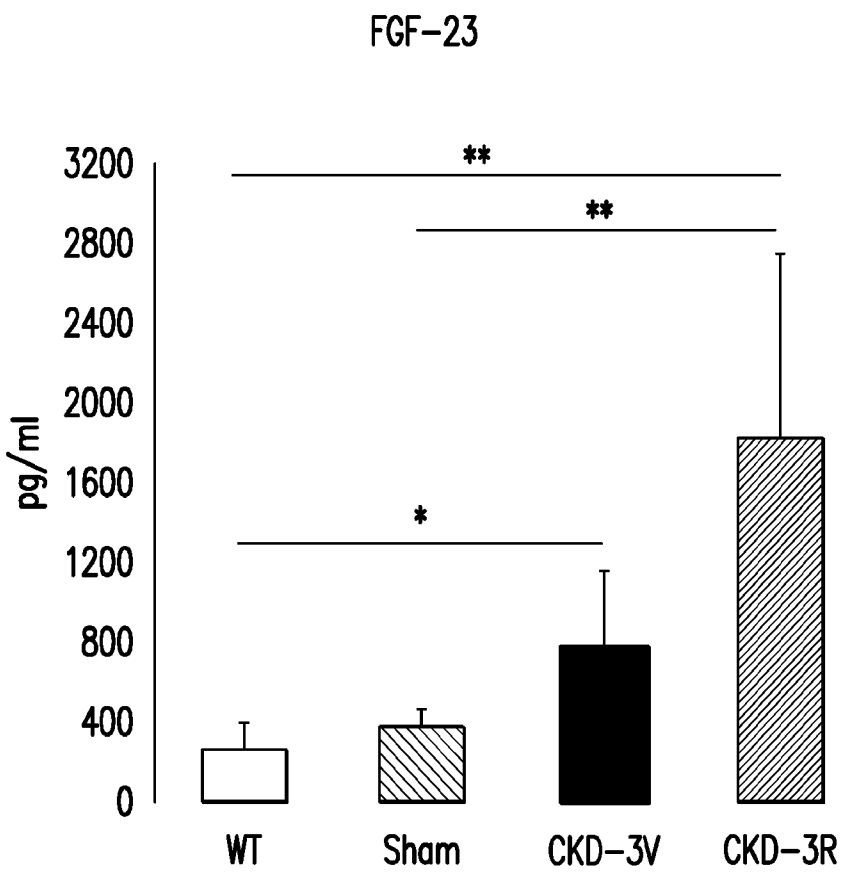

FGF-23
3200
2800
2400
2000
1600
1200
800
400
0
pg/ml
**
**
*
WT
Sham
CKD-3V
CKD-3R

FIG. 20

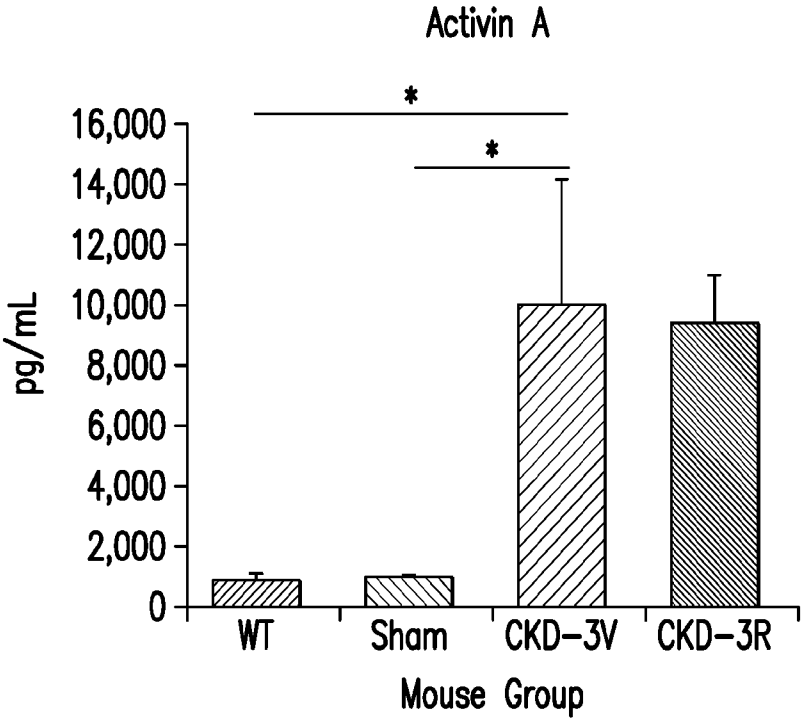


FIG. 21A

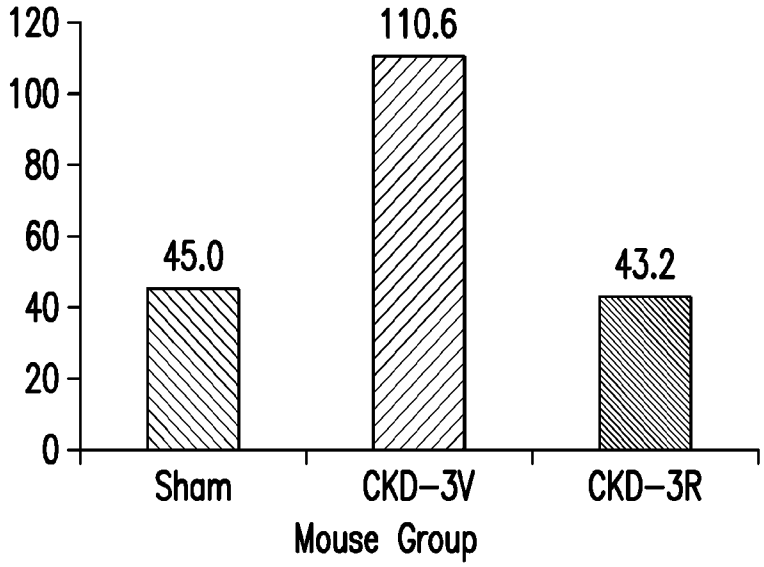


FIG. 21B

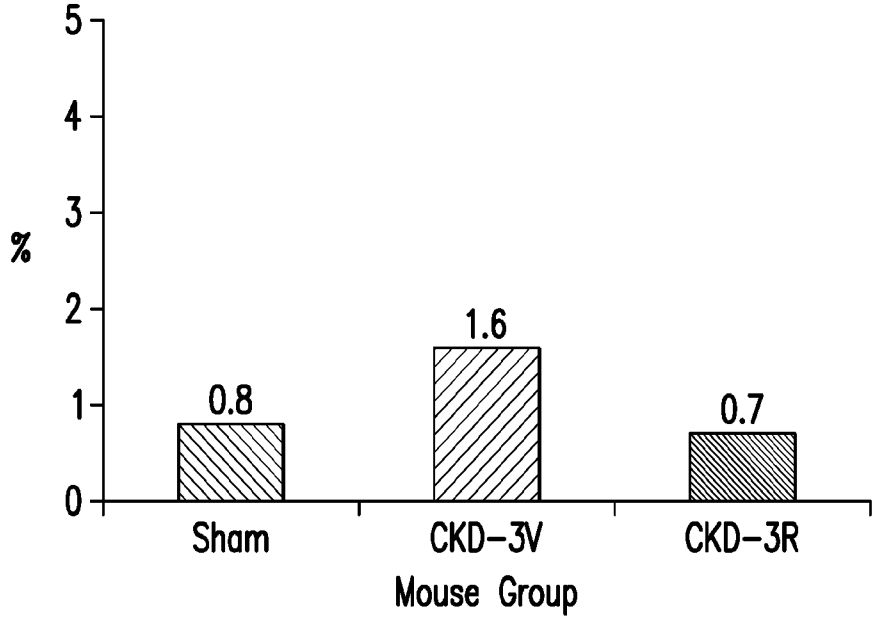


FIG. 21C

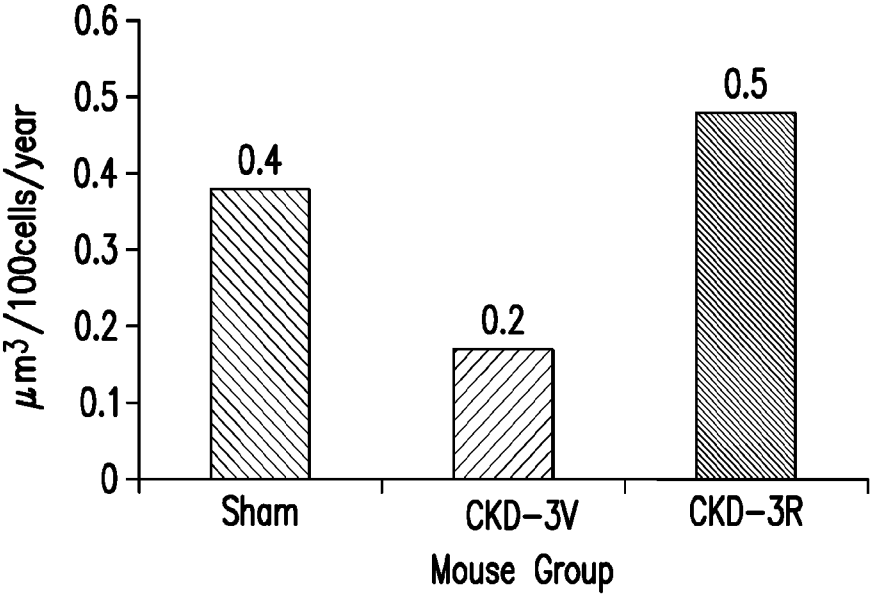
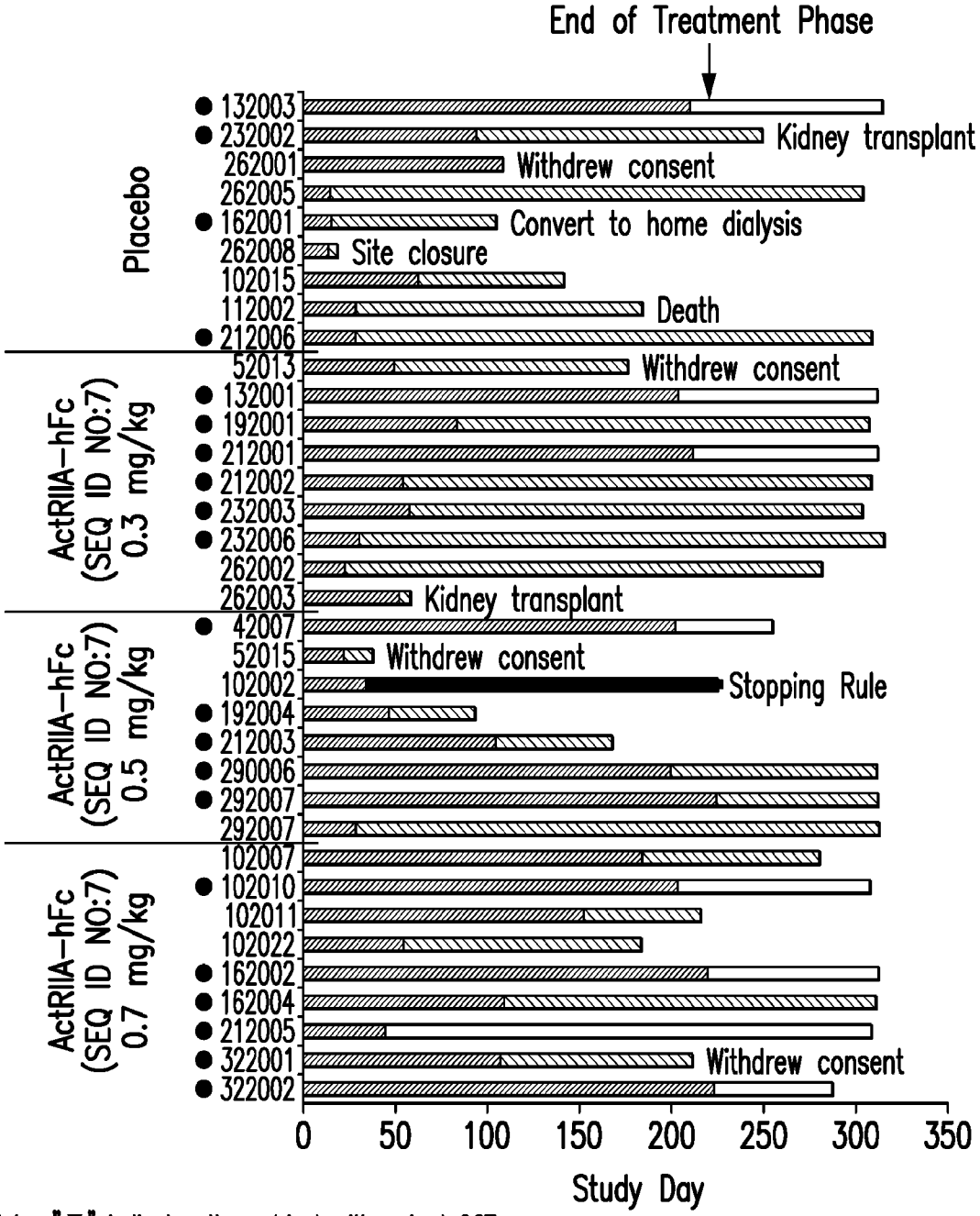


FIG. 21D



Note: "▼" indicates the subject with paired QCT measurements at baseline and Day 225.
*One placebo subject who withdrew consent on Study Day 108 died on Study Day 148; 1 placebo subject who was receiving rescue therapy died on Day 186.
Protocol violation.
Subject met stopping rule criteria for elevated blood on Day 29; study treatment was discontinued, and rescue therapy was administered on Day 36, with continued follow-up.
Subject was randomized in error with non-qualifying blood pressure, based on incomplete evaluation, at baseline.

Days on Study Drug
Days of Follow-up
Days on Rescue Therapy
Stopping Rule

FIG. 22A

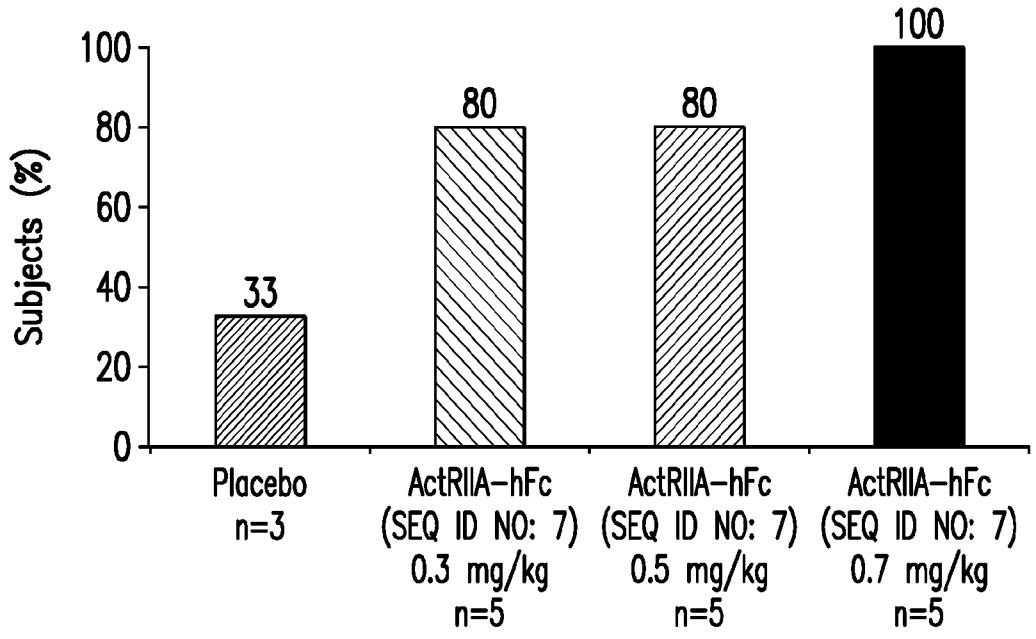


FIG. 22B

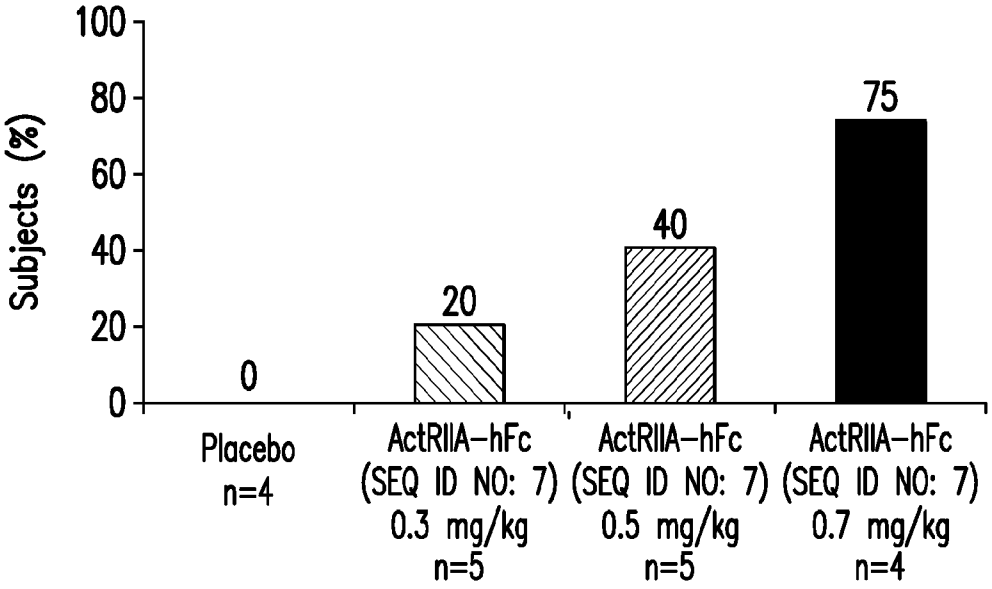


FIG. 22C

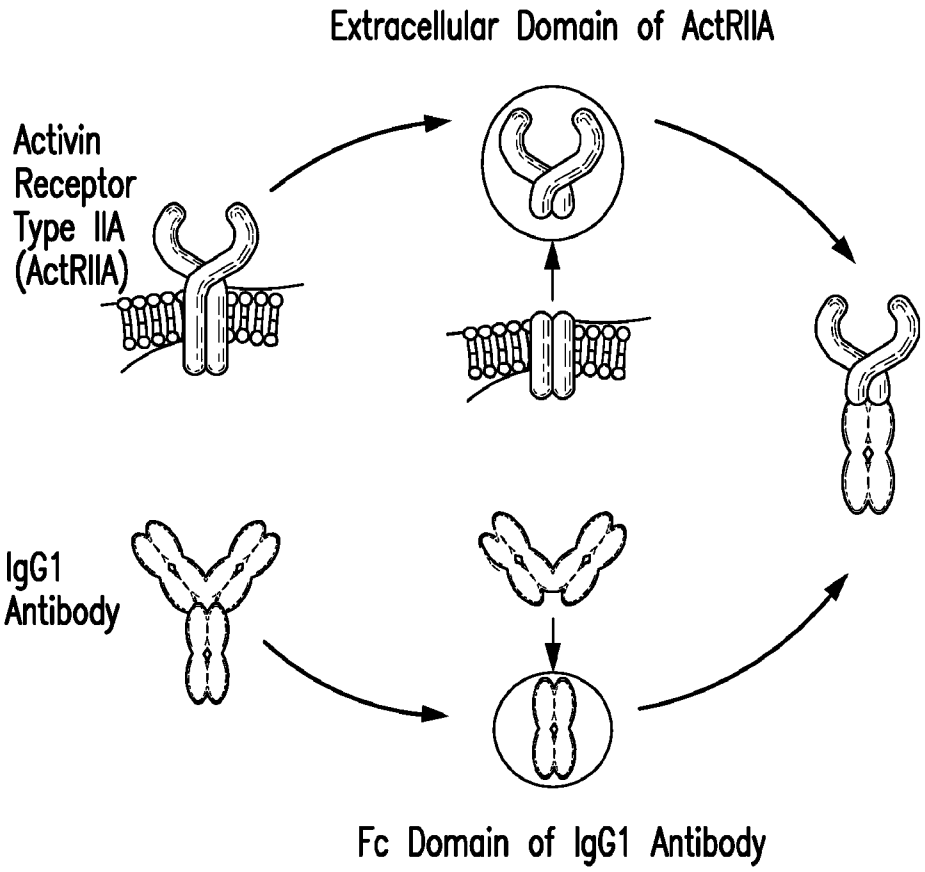


FIG. 23A

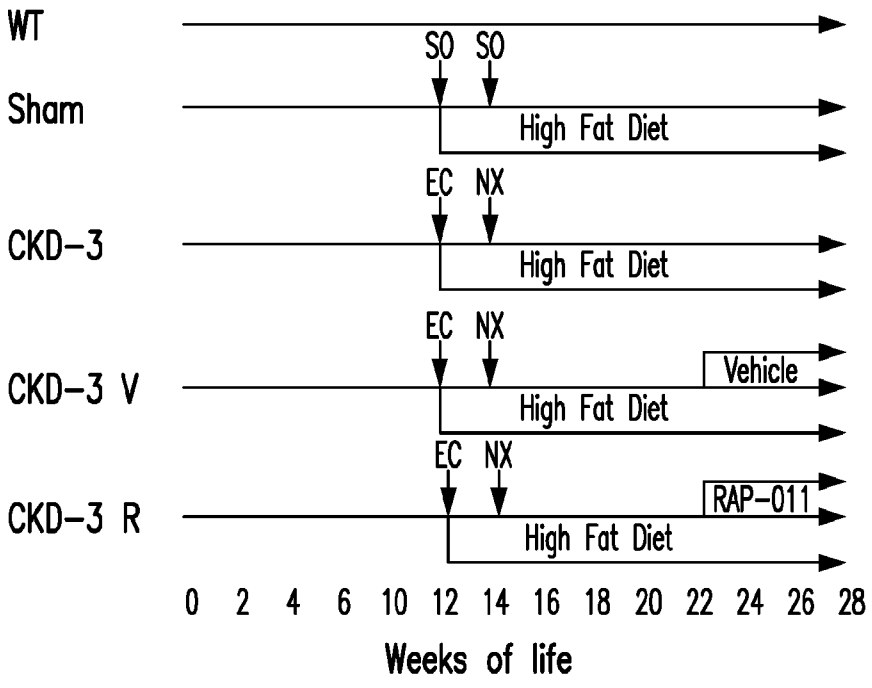


FIG. 23B

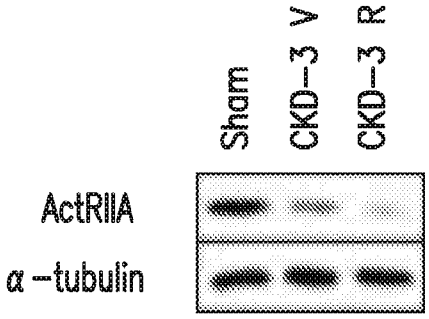


FIG. 24A

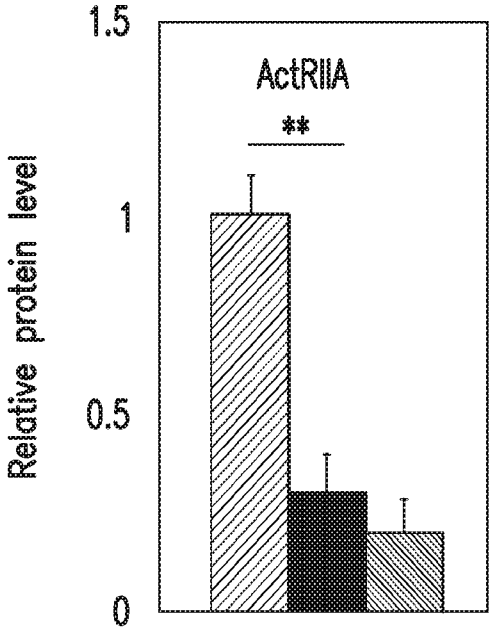


FIG. 24B

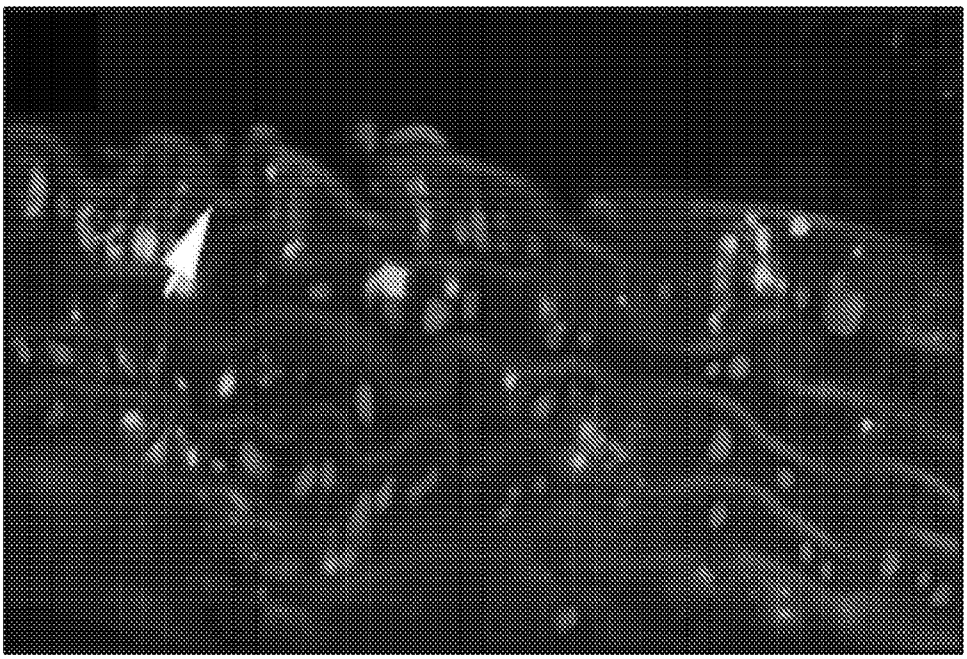


FIG. 24C

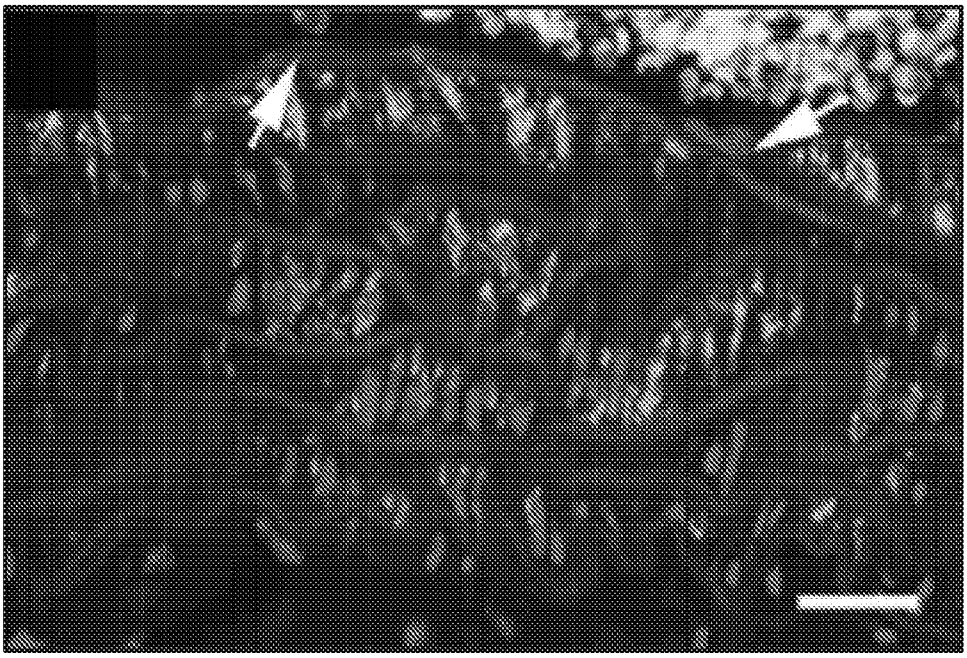


FIG. 24D

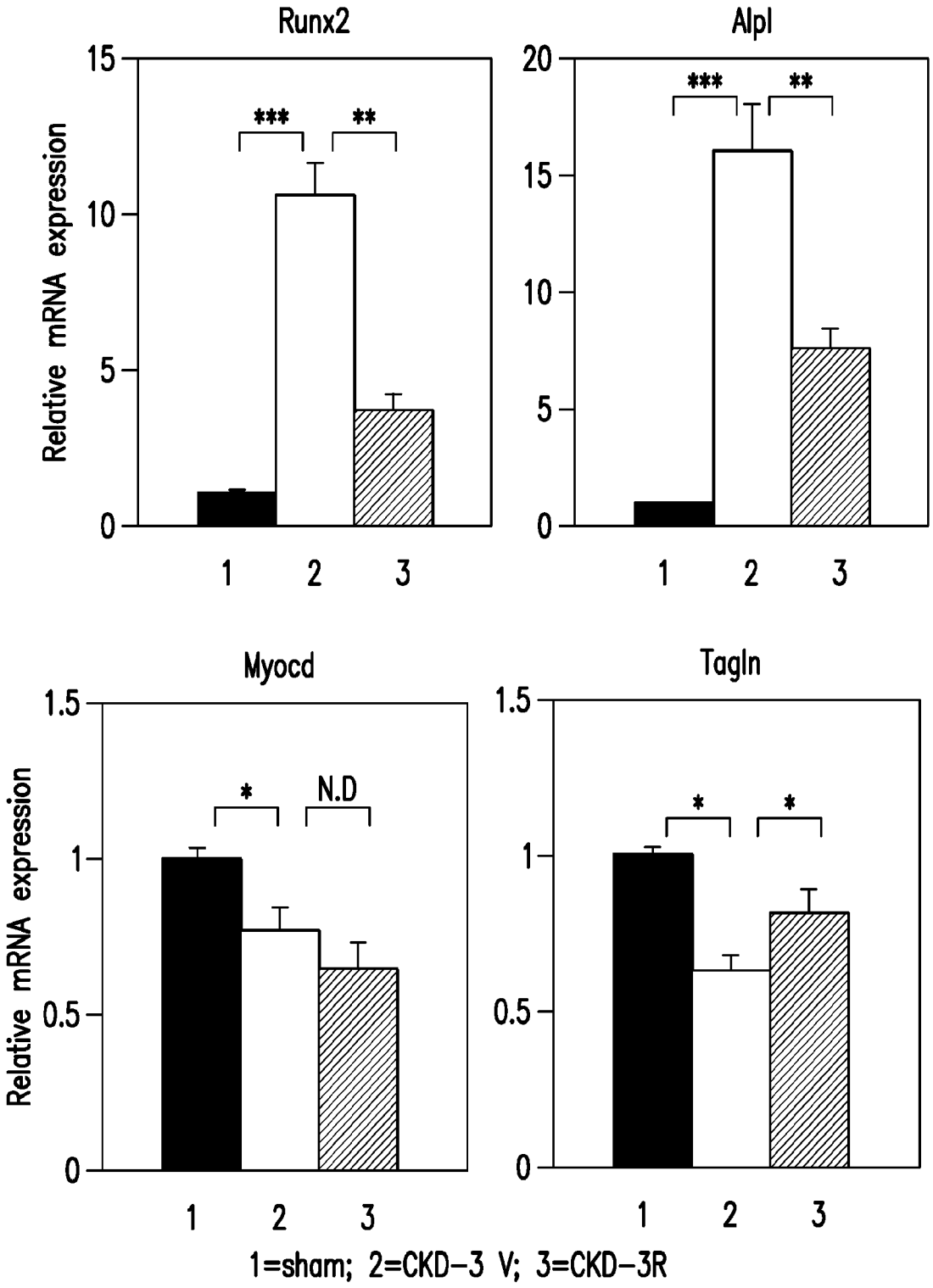

Runx2
Alpl
Myocd
Tagln
Relative mRNA expression
Relative mRNA expression
15
10
5
0
20
15
10
5
0
1.5
1
0.5
0
***
**
*
N.D
1
2
3
1=sham; 2=CKD-3 V; 3=CKD-3R

FIG. 25A

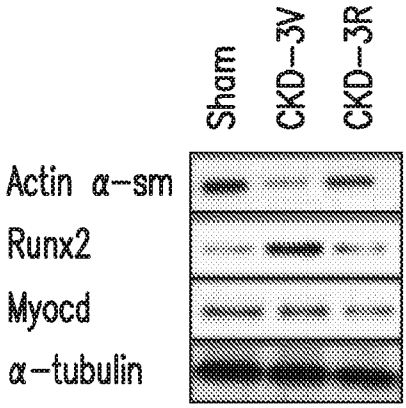


FIG. 25B

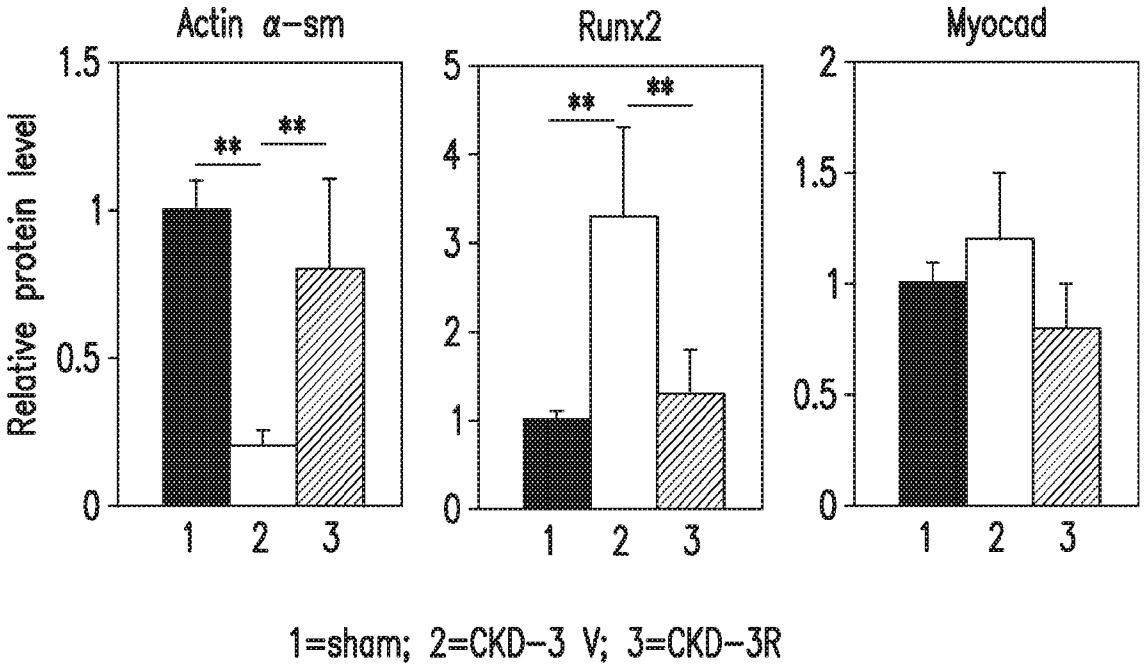
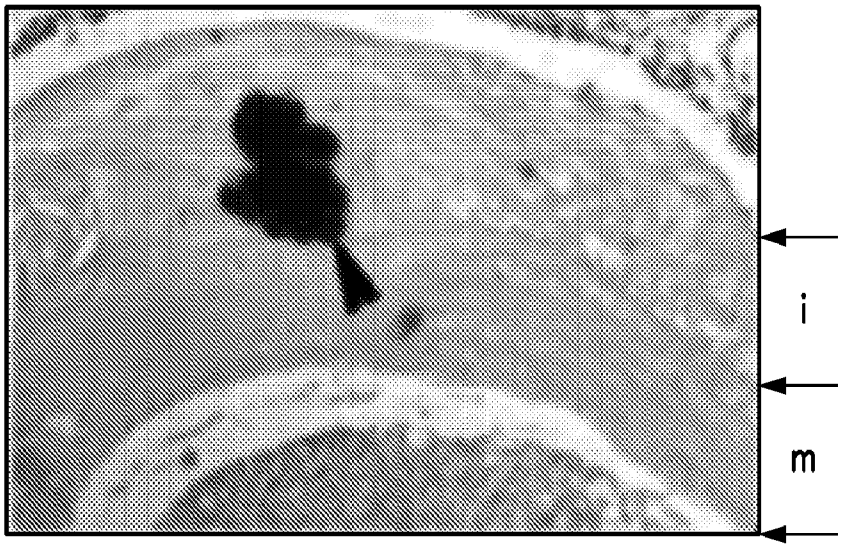
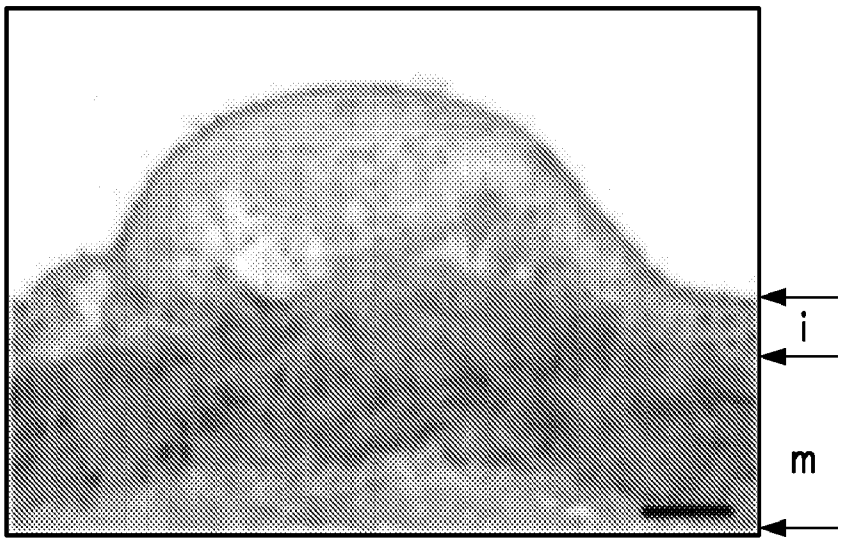


FIG. 25C



CKD-3 V

FIG. 26A



CKD-3 RAP-011

FIG. 26B

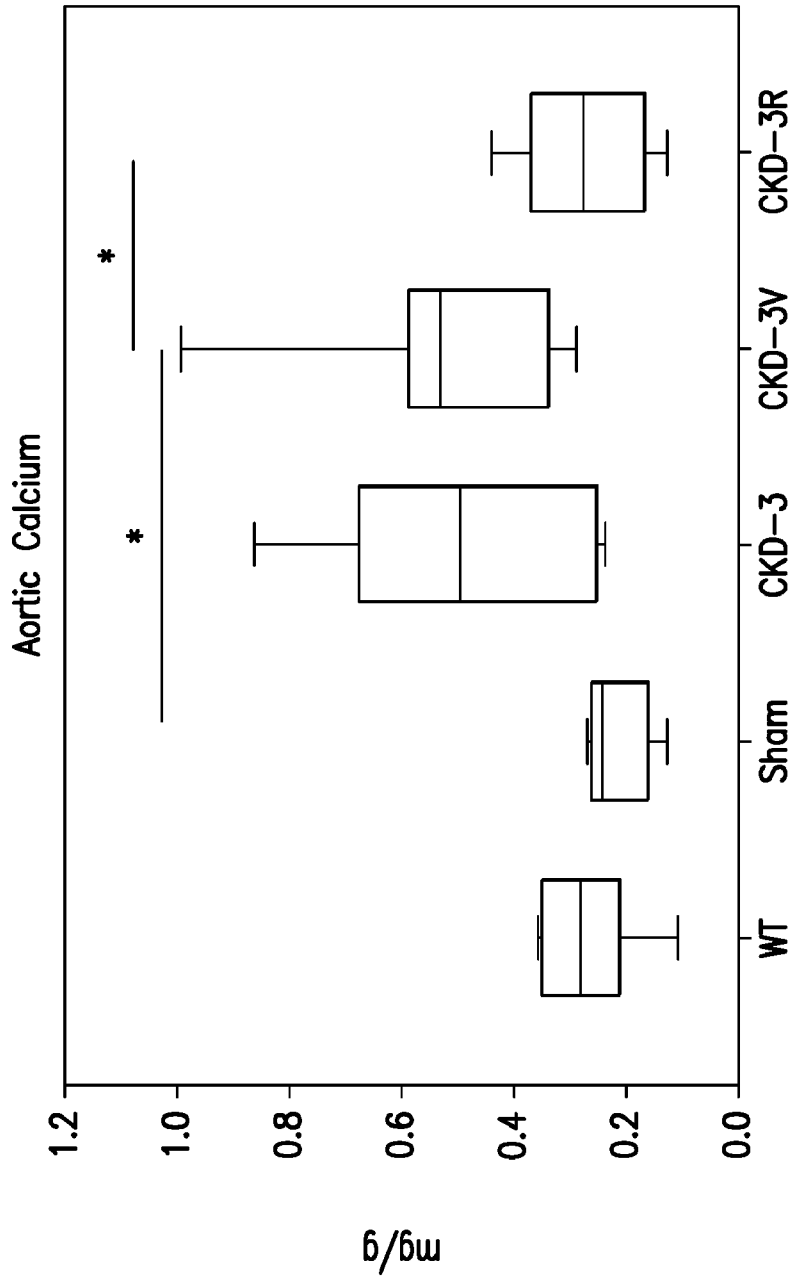

Aortic Calcium
mg/g
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WT
Sham
CKD-3
CKD-3V
CKD-3R
*
*

FIG. 26C

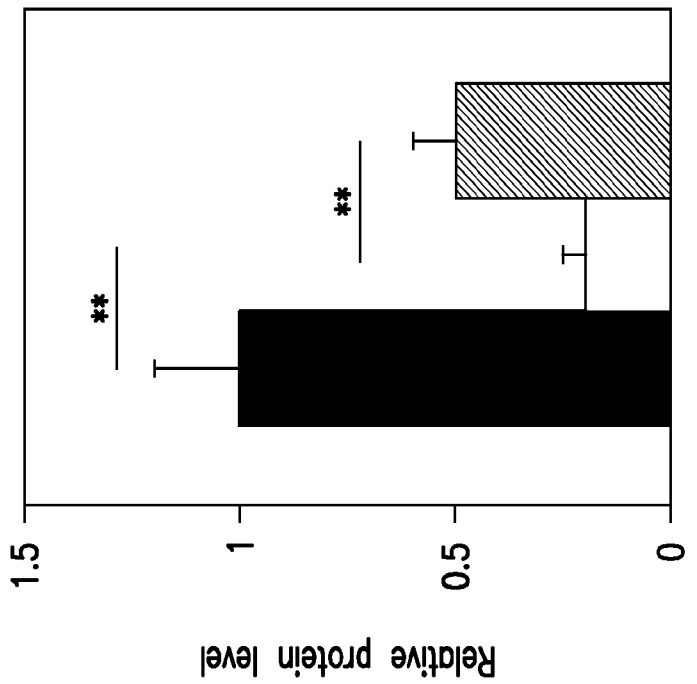


FIG. 27B

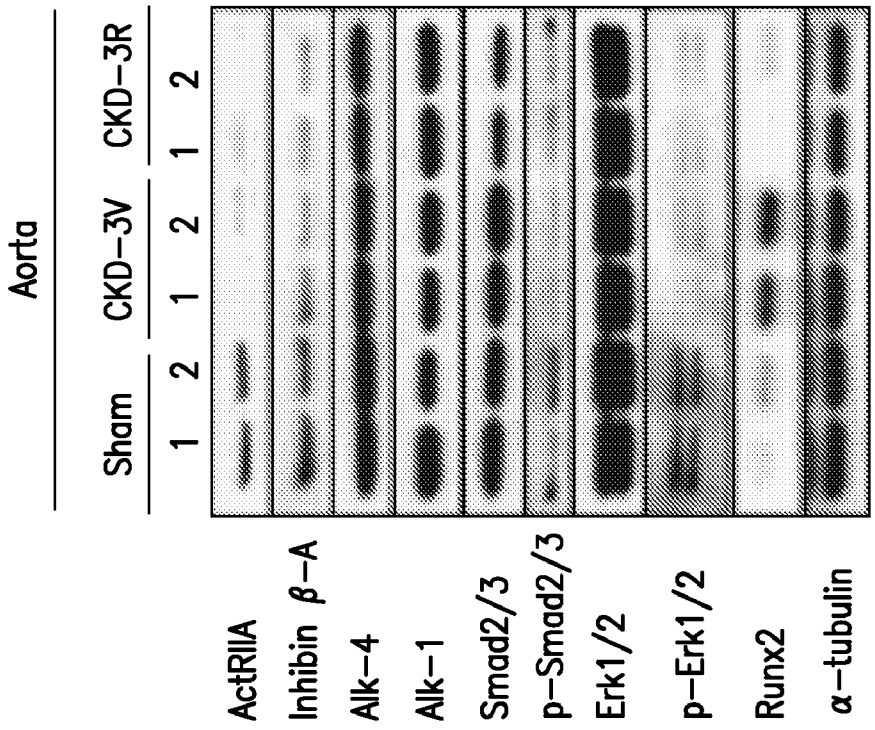
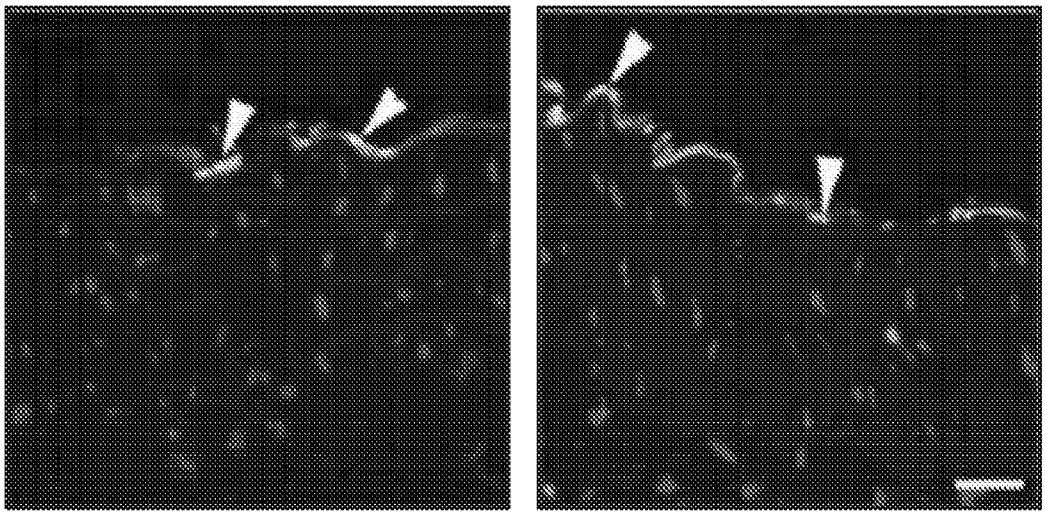


FIG. 27A



DAPI/CD31/beta-catenin

FIG. 28A

FIG. 28B

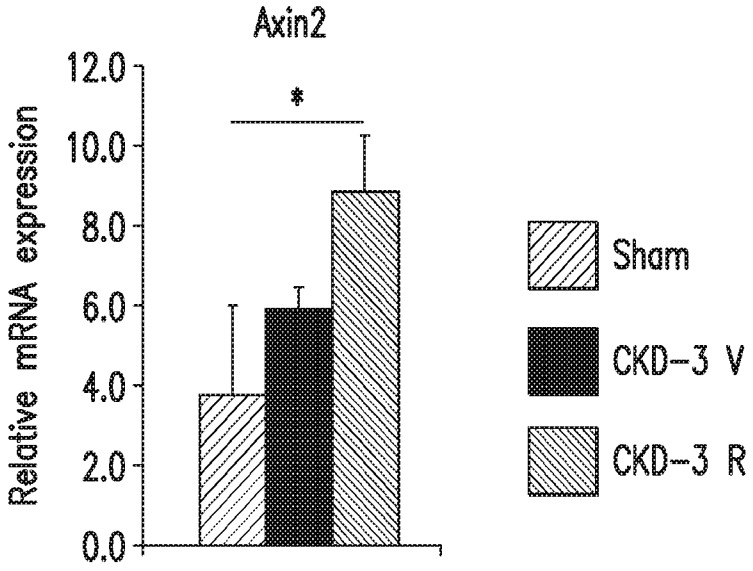


FIG. 28C

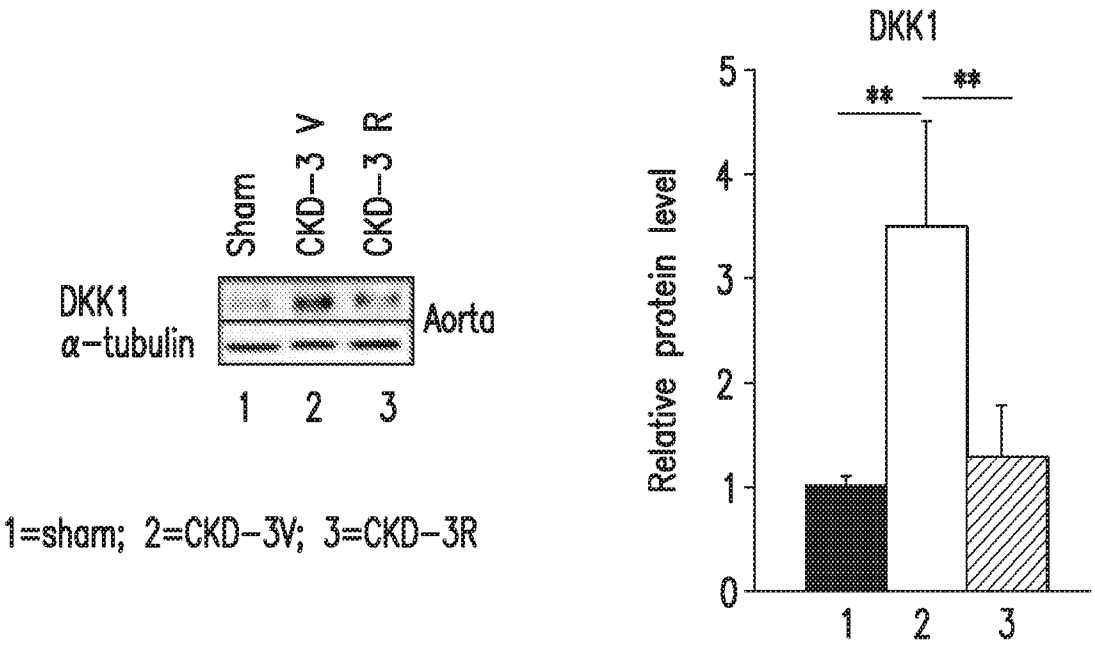


FIG. 28D

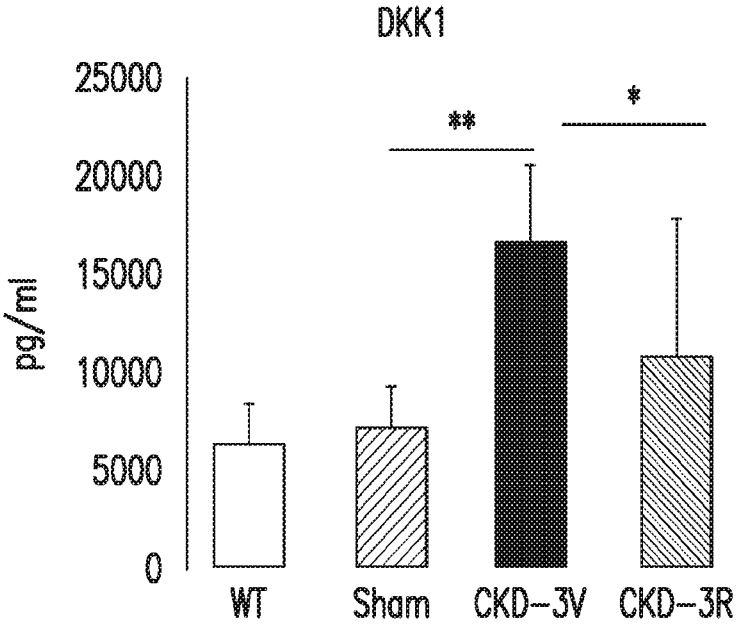


FIG. 28E

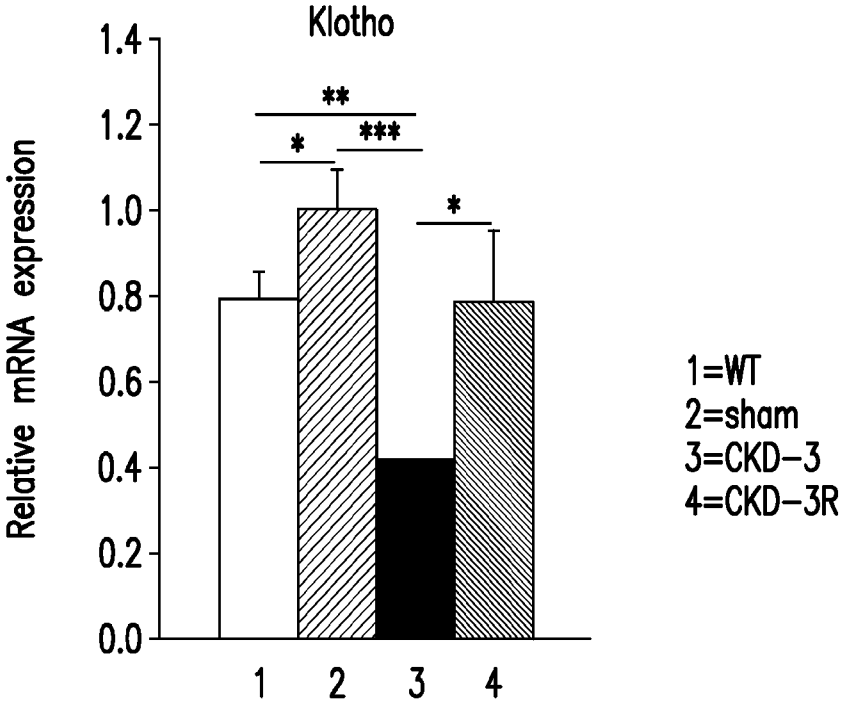


FIG. 29A

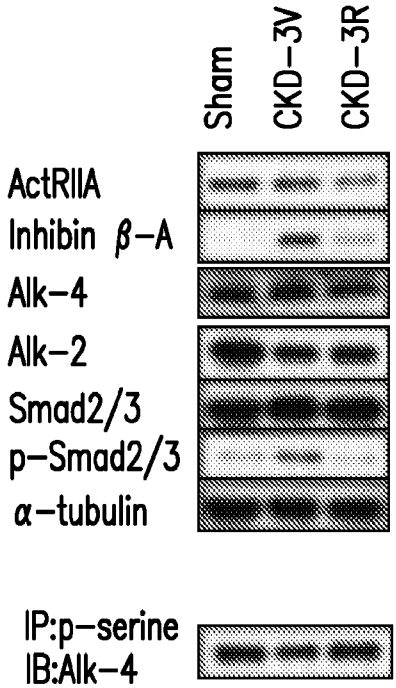


FIG. 29B

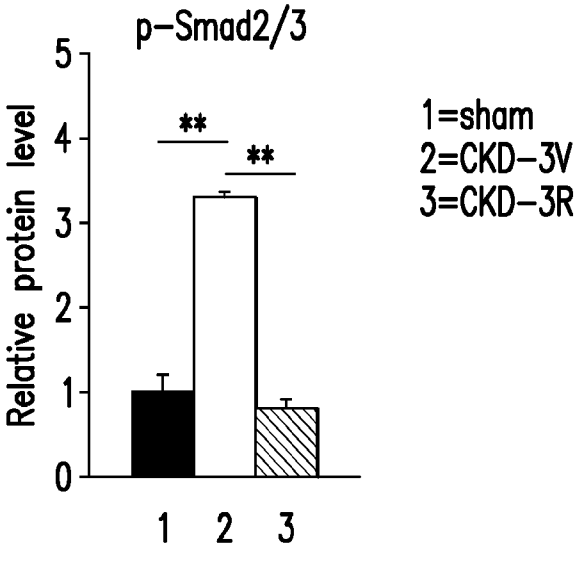


FIG. 29C

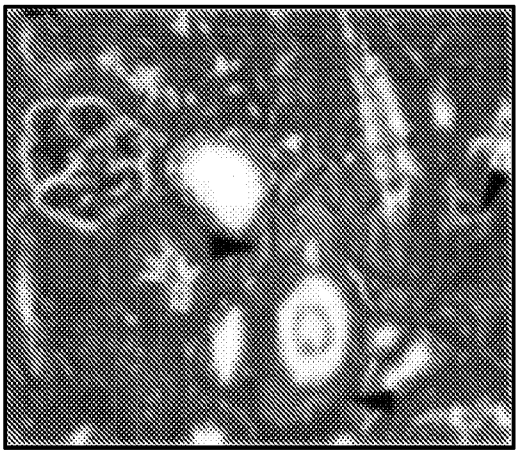


FIG. 30A

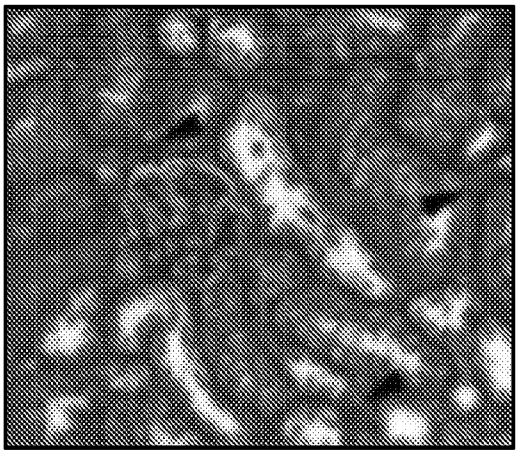


FIG. 30B

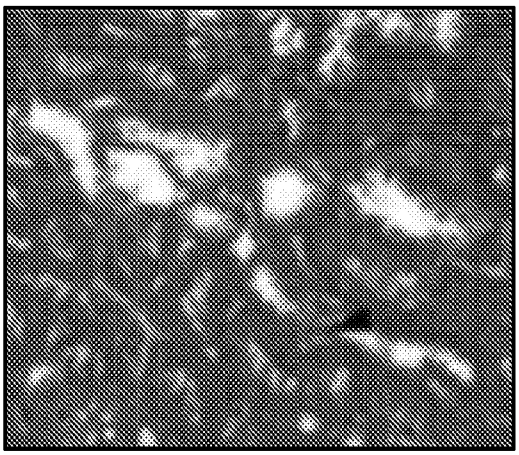


FIG. 30C

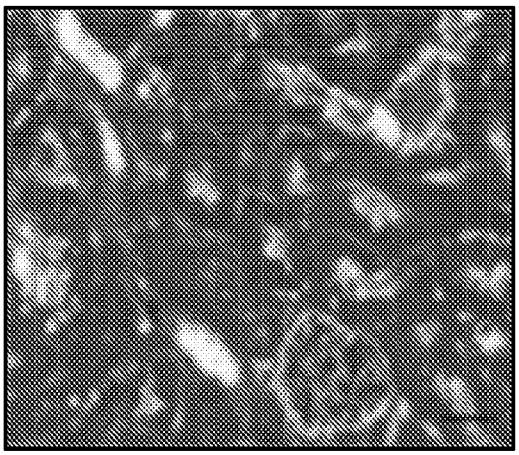


FIG. 30D

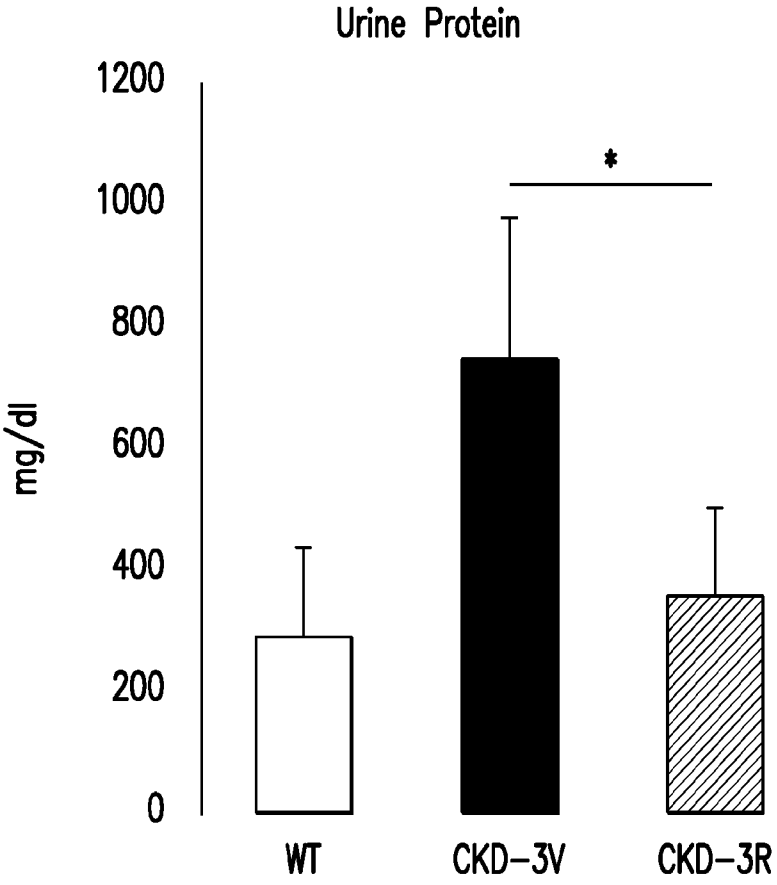


FIG. 30E

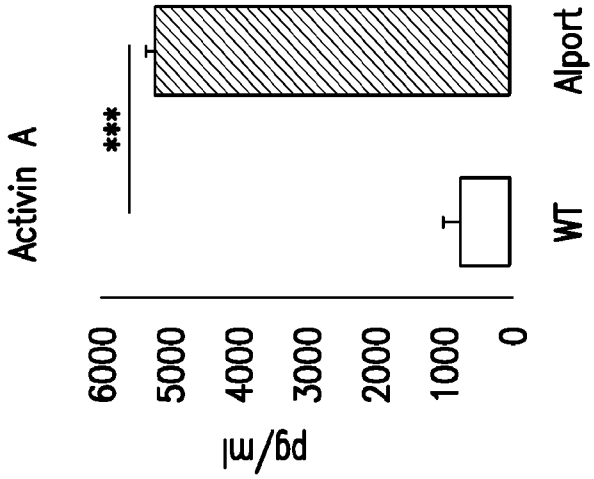


FIG. 31B

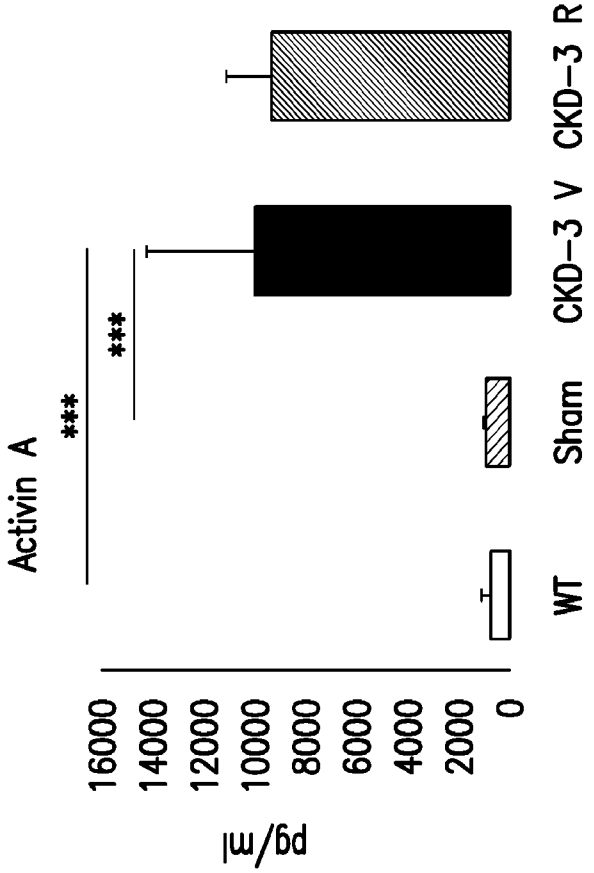


FIG. 31A

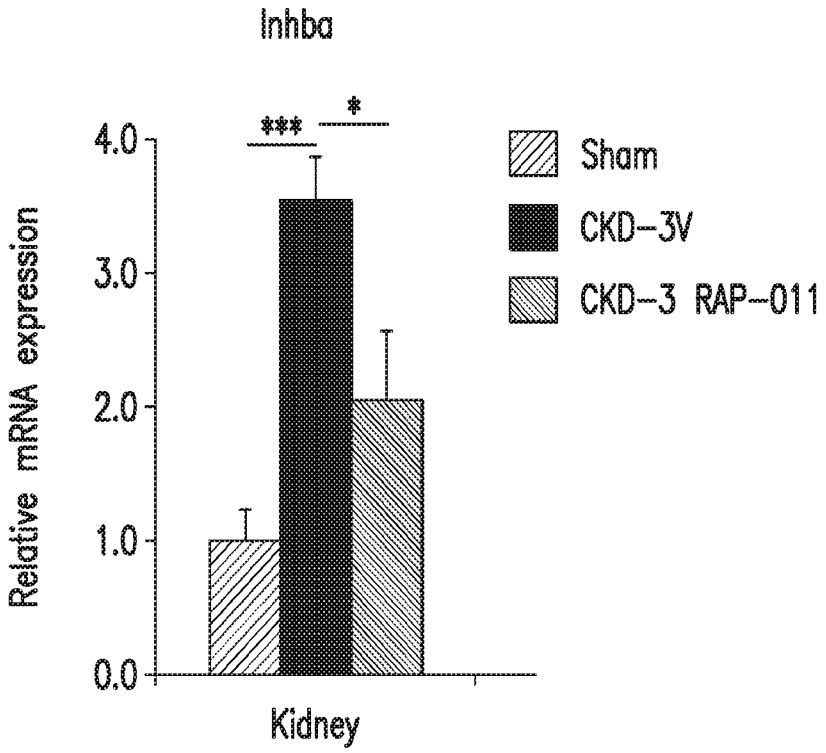


FIG. 31C

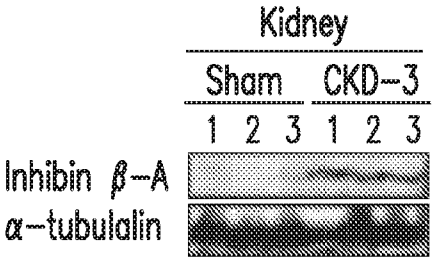


FIG. 31D

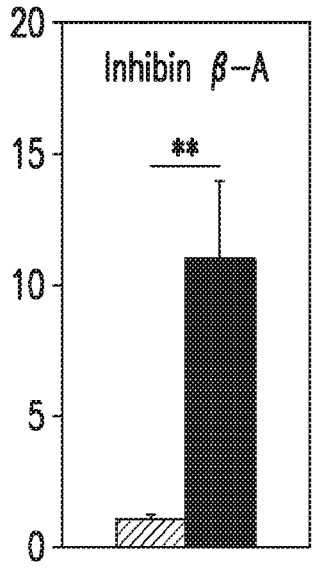


FIG. 31E

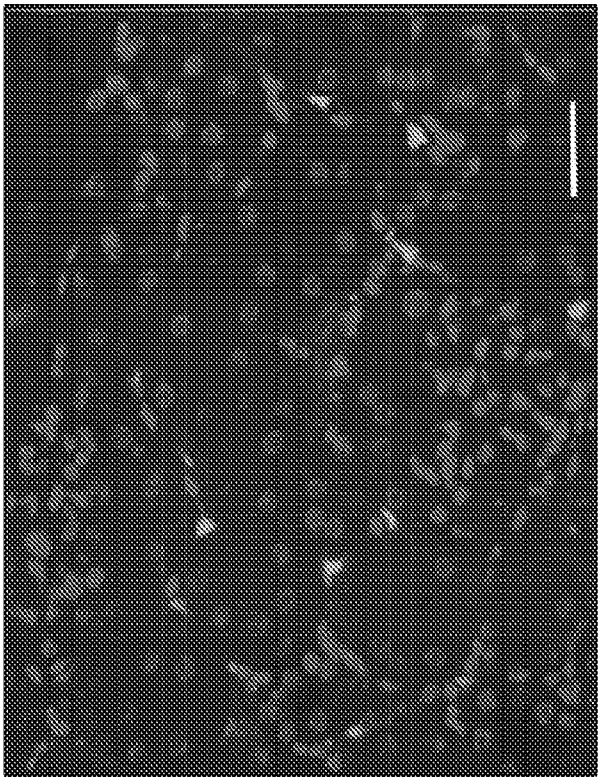


FIG. 32B

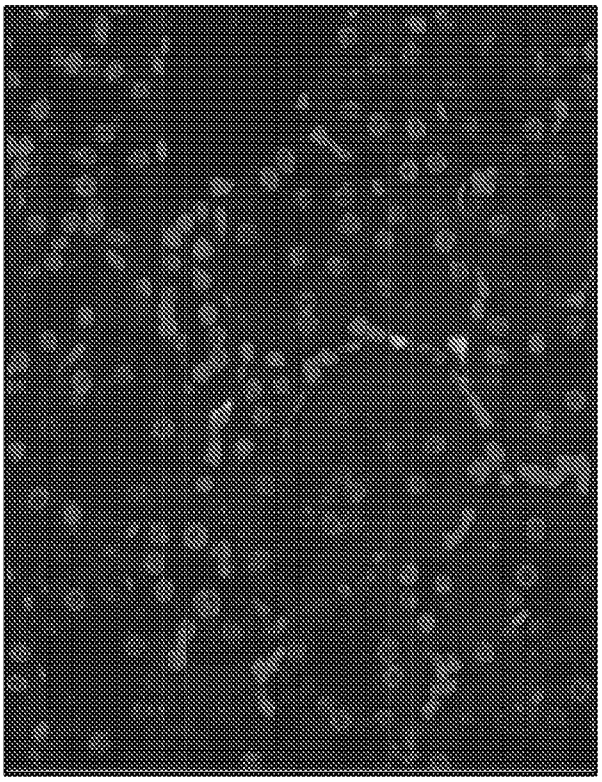


FIG. 32A

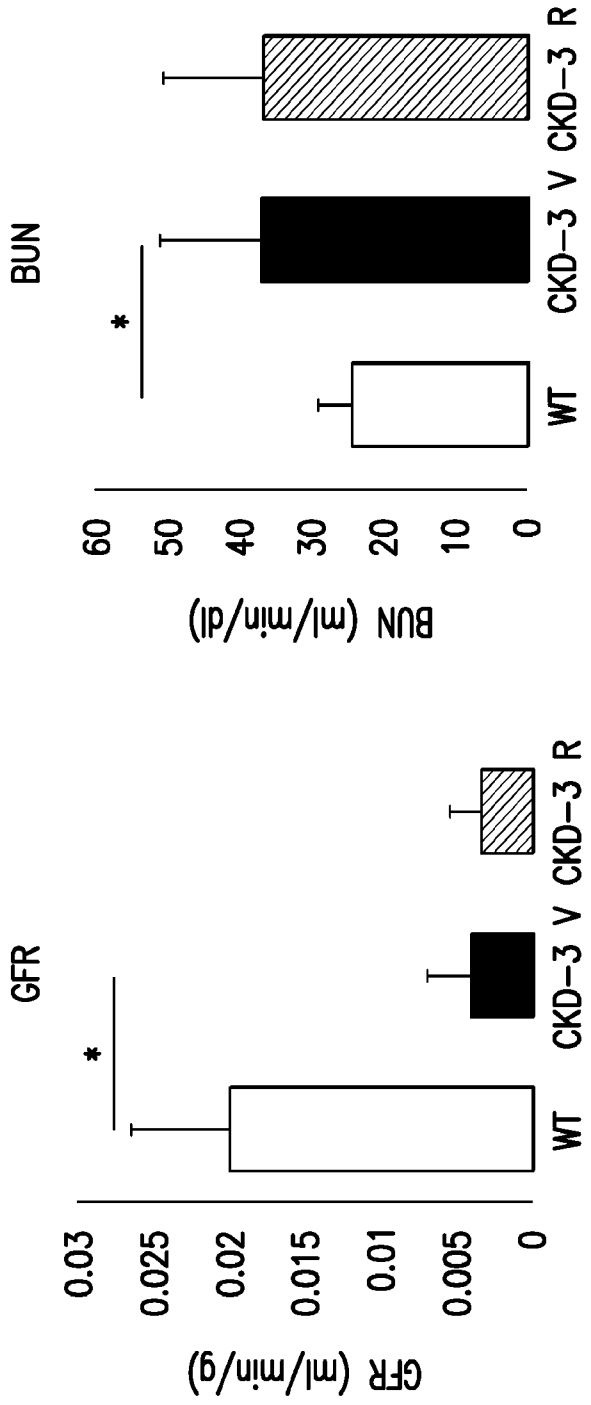

BUN
*
WT
CKD–3 V
CKD–3 R
60
50
40
30
20
10
0
BUN (ml/min/dl)
GFR
*
WT
CKD–3 V
CKD–3 R
0.03
0.025
0.02
0.015
0.01
0.005
0
GFR (ml/min/g)

FIG. 33A

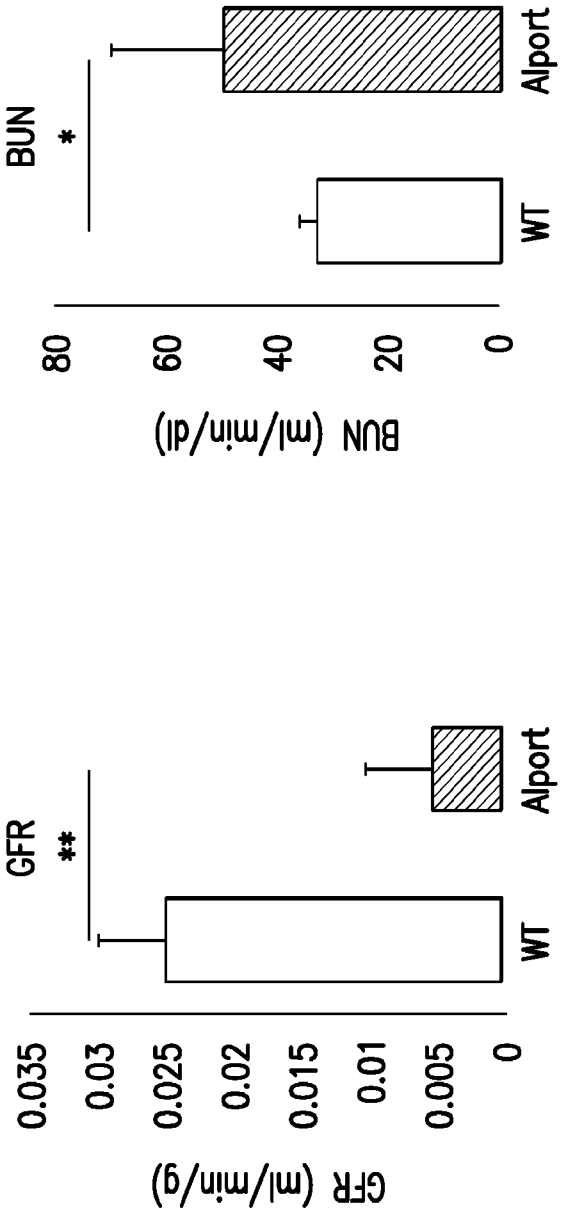

BUN (ml/min/dl)
80
60
40
20
0
BUN
*
WT
Alport
GFR (ml/min/g)
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
GFR
**
WT
Alport

FIG. 33B

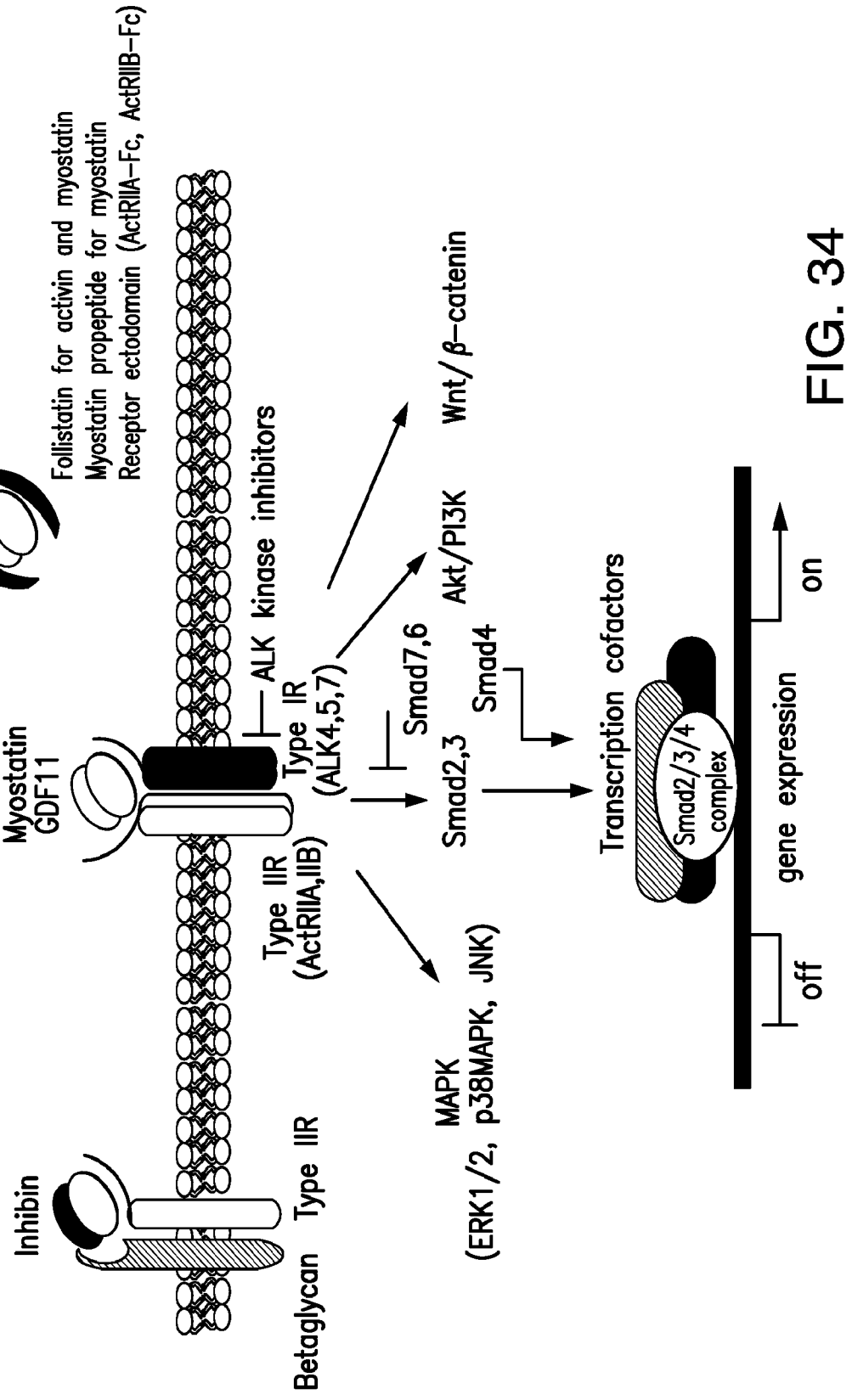

Follistatin for activin and myostatin
Myostatin propeptide for myostatin
Receptor ectodomain (ActRIIA-Fc, ActRIIB-Fc)
ALK kinase inhibitors
Activin
Myostatin
GDF11
Type IR
(ALK4,5,7)
Type IIR
(ActRIIA,IIB)
Inhibin
Betaglycan
Type IIR
Smad7,6
Smad2,3
Smad4
Akt/PI3K
Wnt/β-catenin
MAPK
(ERK1/2, p38MAPK, JNK)
Transcription cofactors
Smad2/3/4
complex
gene expression
on
off

FIG. 34



FIG. 35B

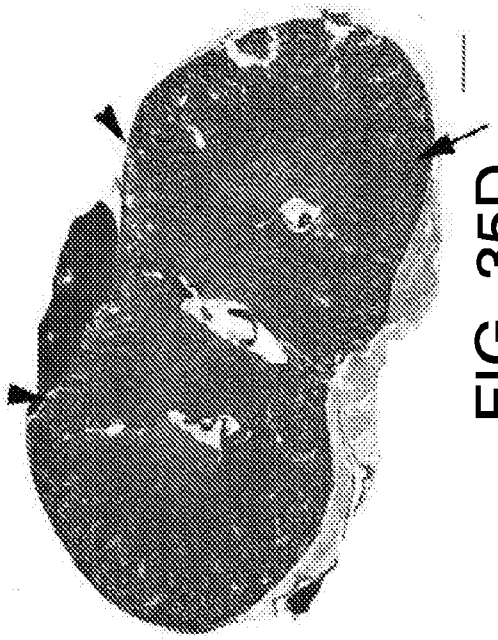


FIG. 35D



FIG. 35A



FIG. 35C

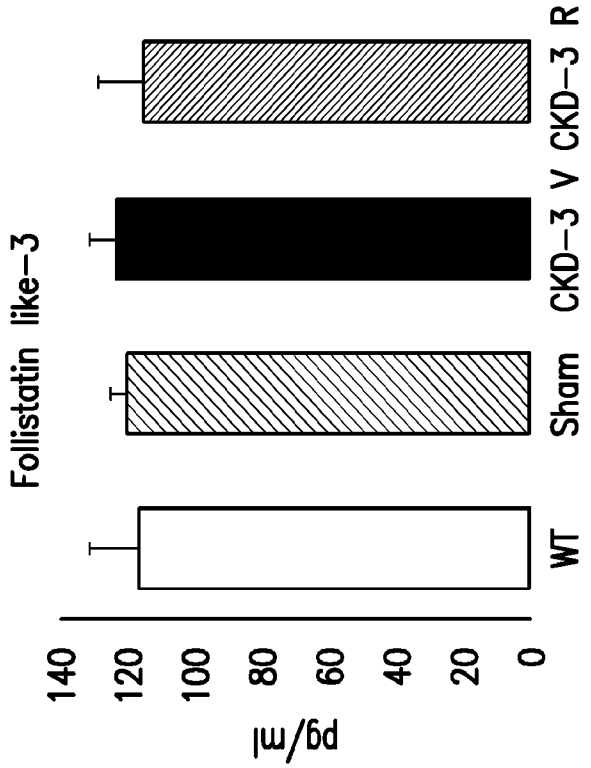


FIG. 36B

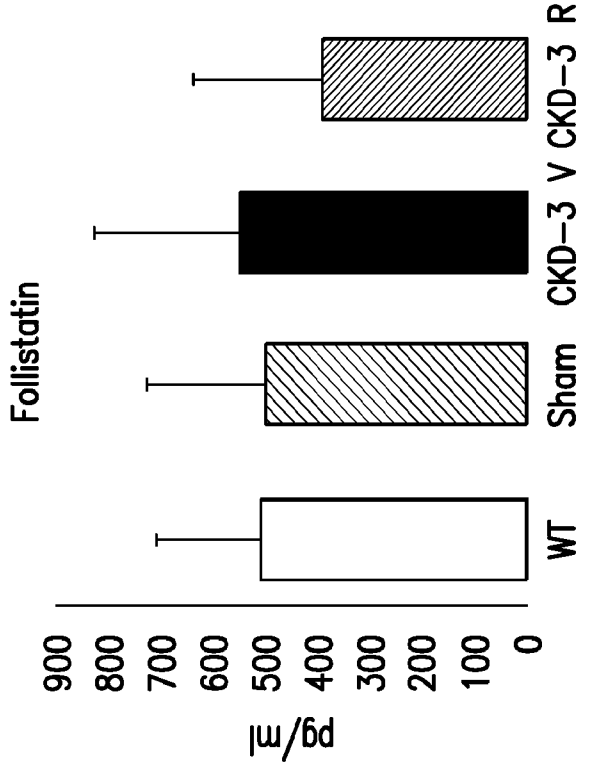


FIG. 36A

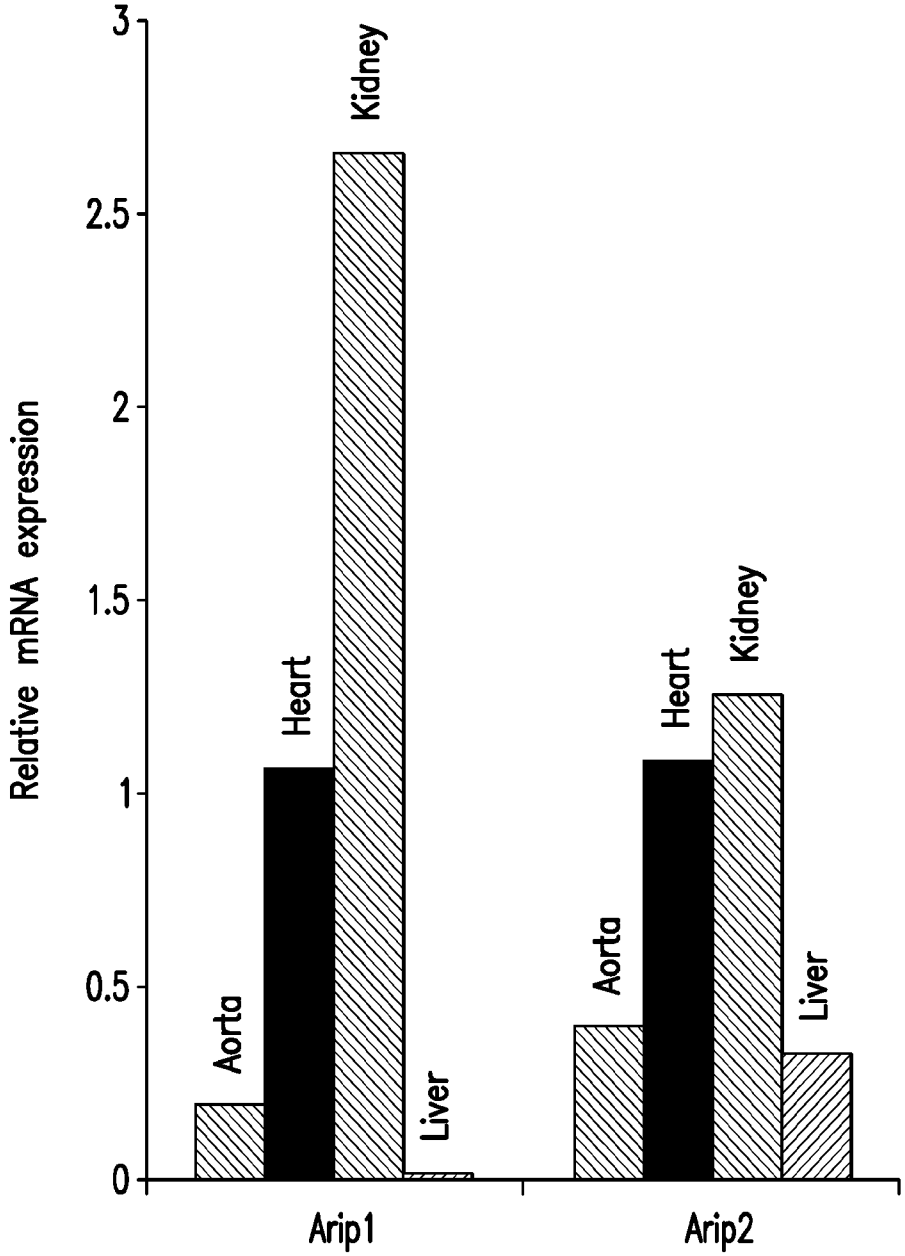

Relative mRNA expression
3
2.5
2
1.5
1
0.5
0
Aorta
Heart
Kidney
Liver
Arip1
Aorta
Heart
Kidney
Liver
Arip2

FIG. 37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/54674

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 9/00, A61K 38/00 (2016.01)
CPC - A61K38/179, C07K14/705
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)
IPC(8): A61P 9/00, A61K 38/00 (2016.01)
CPC: A61K38/179, C07K14/705

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: A61K38/00, G01N2800/52, G06F19/3443

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms: cardiovascular disease, activin type II, ActRII signaling inhibitor activin type II, ActRIIA mRNA, RUNX, SNAIL, activin, DKK1, urinary protein, CTX, klotho, MYOCD, vascular calcification, arterial stiffness, left ventricular hypertrophy (LVH), bone resorption, renal disease, refere

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2014/071158 A1 (CELGENE CORPORATION) 08 May 2014 (08.05.2014) para [0032], [0033], [0035], [0037], [0044], [0047], [0051], [0056], [00206], [00212]	1-10, 14-34, 41-42, 51-52, 63-67
Y	Fang et al. Early chronic kidney disease-mineral bone disorder stimulates vascular calcification. Kidney International (January 2014) Vol 85, No 1, pp 142-150, abstract, pg 142, col 2, para1, pg 143, col 1, para 2, pg 144, col 2, para 1, Fig. 5	1-10, 14-34, 41-42, 51-52, 63-67

☐ Further documents are listed in the continuation of Box C. ☐

* 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
04 February 2016	11 FEB 2016
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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US 15/54674

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☒ on paper or in the form of an image file.

   b. ☒ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/54674

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.: 11-13, 35-40, 43-50, 53-62, 68-80
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

********** See Supplemental Sheet to continue **********

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

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

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

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☒ No protest accompanied the payment of additional search fees.

Continuation of Box No. III, Observations where unity of invention is lacking:

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 1-10, 14-17, (63-67)(in part) directed to a method for treating and/or preventing cardiovascular disease in a subject having altered biomarker levels.

Group II: Claims 18-34, 41-42, 51-52, (63-67)(in part) directed to a method for treating and/or preventing cardiovascular disease in a subject, comprising a process of determining appropriate dosage for the treatment with an ActRII signaling inhibitor.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires a special technical feature of a subject having at least one altered biomarker level as compared to level in a reference population, not required by group II.

Group II require a special technical feature of administering to the subject an adjusted dose of the ActRII signaling inhibitors, not required by group I.

Common Technical Features

The common technical feature shared by Groups I and II is a method for treating and/or preventing a cardiovascular disease in a subject, comprising administering to the subject a pharmaceutically effective dose of an ActRII signaling inhibitor and measuring a level of a biomarker in said subject. The diseases that can be treated with ActRII signaling inhibitor include cardiovascular disease, vascular calcification, arterial stiffness, left ventricular hypertrophy, renal disease, or bone resorption. The biomarker includes, for example, total alkaline phosphatase (ALP) and bone-specific alkaline phosphatase (BSAP).

However, these shared technical feature do not represent a contribution over prior art, because the shared technical feature is anticipated by WO 2014/071158 A1 to Sloan et al. (hereinafter Sloan) (para [0008] " provided herein are methods for treating an adynamic bone disorder in a subject, wherein the method comprises administering a therapeutically effective amount of an ActRII inhibitor to a subject in need.", para [0033] "CKD-MBD can be diagnosed as a systemic disorder of mineral and bone metabolism due to chronic kidney disease and manifested by ... abnormalities of calcium ... alkaline phosphatases (total or bone specific)", para [00172] "Various circulating markers of bone turnover can be used to diagnose bone disorders, such as low bone turnover. Circulating markers of bone turnover are markers of bone formation such as bone specific alkaline phosphatase", para [0042] "In certain embodiments, a subject suffering from, or at risk of suffering from, a form of CKD-MBD and/or extraskeletal calcification, e.g., vascular calcification, has increased levels of FGF23, a hormone produced by osteocytes"; para [0051] "methods described herein result in the improvement of one or more symptoms of vascular calcification. Exemplary symptoms include, without limitation, increases in the levels of vascular (e.g., arterial) calcium, ... loss of arterial elasticity, ... development of left ventricular hypertrophy, decrease in coronary artery perfusion, and myocardial ischaemia.", para [0026] Figure 6: mActRIIA-Fc increases Bone Mineral Density.", para [0019] "In certain embodiments, the subject to be treated with the methods provided herein has end stage renal disease.")

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

continuation of item no. 4: Claims 11-13, 35-40, 43-50, 53-62, 68-80 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(71)申请人 细胞基因公司
地址 美国新泽西州
申请人 华盛顿大学

(72)发明人 基思·鲁斯卡 方一夫
威廉姆·史密斯 陈念航

(74)专利代理机构 北京安信方达知识产权代理有限公司 11262
代理人 郑霞

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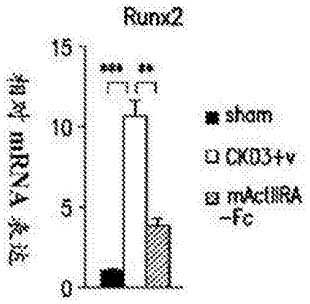
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(54)发明名称

利用ACTRII配体陷阱治疗心血管疾病

(57)摘要

本文提供的是治疗对象的与血管钙化和/或心血管疾病和/或骨吸收相关的疾病的方法，通过使用生物标志物的水平，特别是snail同源物1(Snail)、phosphosmad2、phosphosmad3、尿蛋白、dickkopf同源物1(Dkk1)、1型胶原蛋白alpha1(Col1a1)、激活素(例如，游离激活素)、runt-相关的转录因子2(Runx2)、碱性磷酸酶(Alp)、骨特异性碱性磷酸酶(BSAP)、C-末端1型胶原蛋白端肽(CTX)、osterix、Klotho、alpha-平滑肌肌动蛋白(alpha-SMA)、心肌素(MYOCD)、激活素2A型受体(ActRIIA)、轴抑制蛋白2(Axin2)和/或平滑肌蛋白质22-alpha(Sm22-alpha)，作为用激活素II型受体信号转导抑制剂治疗的对象对治疗的响应、治疗效果或适当剂量的指示。



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1.一种治疗和/或预防对象的心血管疾病的方法,包括向所述对象施用药学上有效剂量的II型激活素受体(ActRII)信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的runt相关转录因子2(Runx2)水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的碱性磷酸酶(Alp)水平;

(c)与参考群体中snail同源物1(Snai1)水平相比提高的Snai1水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的dickkopf同源物1(Dkk1)水平相比提高的Dkk1水平;

(f)与参考群体中的1型胶原蛋白alpha1(col1a1)水平细胞提高的col1a1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的骨特异性碱性磷酸酶(BSAP)水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha平滑肌肌动蛋白(alpha-SMA)水平相比降低的alpha-SMA水平;

(m)与参考群体中的心肌素(MYOCD)水平相比降低的MYOCD水平;

(n)与参考群体中的平滑肌蛋白22-alpha(Sm22-alpha)水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

2.一种治疗和/或预防对象的血管钙化的方法,包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的Runx2水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的Alp水平;

(c)与参考群体中的Snai1水平相比提高的Snai1水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;

(f)与参考群体中的col1a1水平相比提高的col1a1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的BSAP水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;

(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;

(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

3.一种治疗和/或预防对象的心血管疾病的方法,包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的Runx2水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的Alp水平;

(c)与参考群体中的Snail水平相比提高的Snail水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;

(f)与参考群体中的colla1水平相比提高的colla1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的BSAP水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;

(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;和/或

(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平;和

其中所述心血管疾病与血管钙化相关和/或由血管钙化引起。

4.一种治疗和/或预防对象的动脉硬化水平升高的方法,包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的Runx2水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的Alp水平;

(c)与参考群体中的Snail水平相比提高的Snail水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;

(f)与参考群体中的colla1水平相比提高的colla1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的BSAP水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;

(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;和/或

(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

5.一种治疗和/或预防对象的心血管疾病的方法,包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的Runx2水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的Alp水平;

(c)与参考群体中的Snail水平相比提高的Snail水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;

(f)与参考群体中的colla1水平相比提高的colla1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的BSAP水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;

(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;和/或

(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平,

其中所述心血管疾病与动脉硬化水平升高相关和/或由动脉硬化水平升高引起。

6.一种治疗和/或预防对象的左心室肥大(LVH)的方法,包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的Runx2水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的Alp水平;

(c)与参考群体中的Snail水平相比提高的Snail水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;

(f)与参考群体中的colla1水平相比提高的colla1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的BSAP水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;

(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;和/或

(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

7.一种治疗和/或预防对象的心血管疾病的方法,包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的Runx2水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的Alp水平;

(c)与参考群体中的Snail水平相比提高的Snail水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;

(f)与参考群体中的colla1水平相比提高的colla1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的BSAP水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;

(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;和/或

(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平;

其中所述心血管疾病与左心室肥大相关和/或由左心室肥大引起。

8.一种治疗和/或预防对象的心血管疾病的方法,包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的Runx2水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的Alp水平;

(c)与参考群体中的Snail水平相比提高的Snail水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;

(f)与参考群体中的colla1水平相比提高的colla1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的BSAP水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;

(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;和/或

(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平;和

以及其中所述心血管疾病与肾病相关和/或由肾病引起。

9.一种降低对象的骨吸收的方法,包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂,其中所述对象具有:

(a)与参考群体中的Runx2水平相比提高的Runx2水平;

(b)与参考群体中的Alp水平相比提高的Alp水平;

(c)与参考群体中的Snai1水平相比提高的Snai1水平;

(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;

(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;

(f)与参考群体中的colla1水平相比提高的colla1水平;

(g)与参考群体中的激活素水平相比提高的激活素水平;

(h)与参考群体中的BSAP水平相比提高的BSAP水平;

(i)与参考群体中的CTX水平相比提高的CTX水平;

(j)与参考群体中的Osterix水平相比提高的Osterix水平;

(k)与参考群体中的Klotho水平相比降低的Klotho水平;

(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;

(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;

(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;

(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;

(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或

(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

10.权利要求1-9的任一项的方法,其中药学上有效剂量的ActRII信号转导抑制剂是约15mg、约30mg、约45mg、约60mg、约75mg、约90mg或约1g或约0.1mg/kg、约0.13mg/kg、约0.2mg/kg、约0.26mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg。

11.权利要求1-10的任一项的方法,其中所述药学上有效的剂量通过注射施用。

12.权利要求1-11的任一项的方法,其中所述药学上有效的剂量(i)每14天施用一次;(ii)每21天施用一次;(iii)每28天施用一次,或(iv)每42天施用一次。

13.权利要求1-11的任一项的方法,其中所述药学上有效的剂量连续地和/或无限地施用。

14.权利要求1-9的任一项的方法,其中所述Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的提高的水平分别高于参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的水平约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%、100%、200%或500%。

15.权利要求1-9的任一项的方法,其中所述Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的提高的水平等于或约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%、100%、200%或500%高于参考

群体的前10%、前5%、前4%、前3%、前2%或前1%中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的水平。

16.权利要求1-9的任一项的方法，其中所述Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的降低的水平分别低于参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%或100%。

17.权利要求1-9的任一项的方法，其中所述Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的降低的水平等于或约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%或100%低于参考群体的后10%、后5%、后4%、后3%、后2%或后1%中Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平。

18.一种治疗和/或预防对象的血管钙化的方法，其中所述方法包括：

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象；

(b)进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量；

(c)一段时间以后，进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量；和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂，其中所述第一次测量在治疗的开始之前，或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行，以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化，从而治疗和/或预防所述血管钙化。

19.一种治疗和/或预防对象的心血管疾病的方法，其中所述方法包括：

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象；

(b)进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量；

(c)一段时间以后，进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量；和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂，其中所述第一次测量在治疗的开始之前，或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行，以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化，从而治疗和/或预防所述心血管疾病。

20.一种治疗和/或预防对象的心血管疾病的方法，其中所述方法包括：

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象；

(b)进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量；

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述心血管疾病与血管钙化相关和/或由血管钙化引起,其中所述第一次测量在治疗的开始之前,或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

21.一种治疗和/或预防对象的心血管疾病的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述心血管疾病与肾病相关和/或由肾病引起;其中所述第一次测量在治疗的开始之前,或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

22.一种治疗和/或预防对象的心血管疾病的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述心血管疾病与动脉硬化水平升高相关和/或由动脉硬化水平升高引起;其中所述第一次测量在治疗的开始之前,或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

23.一种治疗和/或预防对象的心血管疾病的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述心血管疾病与左心室肥大相关和/或由左心室肥大引起;其中所述第一次测量在治疗的开始之前,或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

24.一种降低对象的骨吸收的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述第一次测量在治疗的开始之前,或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而降低所述骨吸收。

25.一种治疗和/或预防对象的血管钙化的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述血管钙化。

26.一种治疗和/或预防对象的动脉硬化水平升高的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述动脉硬化水平升高。

27.一种治疗和/或预防对象的心血管疾病的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述心血管疾病与动脉硬化水平升高相关和/或由动脉硬化水平升高引起,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

28.一种治疗和/或预防对象的左心室肥大(LVH)的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述LVH。

29.一种治疗和/或预防对象的心血管疾病的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述心血管疾病与左心室肥大相关和/或由左心室肥大引起,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

30.一种治疗和/或预防对象的心血管疾病的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

31.一种治疗和/或预防对象的心血管疾病的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂;

其中所述心血管疾病与血管钙化相关和/或由血管钙化引起,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

32.一种治疗和/或预防对象的心血管疾病的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂;

其中所述心血管疾病与肾病相关和/或由肾病引起,以及其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而治疗和/或预防所述心血管疾病。

33.一种降低对象的骨吸收的方法,其中所述方法包括:

(a)向所述对象施用初始剂量的ActRII信号转导抑制剂给所述对象;

(b)进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、coll a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;

(c)一段时间以后,进行所述对象的Snail、phosphosmad2、phosphosmad3、尿蛋白、

Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和

(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂,其中所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化,从而降低所述骨吸收。

34.权利要求18-33的任一项的方法,其中所述ActRII信号转导抑制剂的初始剂量是约15mg、约30mg、约45mg、约60mg、约75mg、约330mg或约1g或约0.1mg/kg、约0.13mg/kg、约0.2mg/kg、约0.26mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.33mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg。

35.权利要求18-34的任一项的方法,其中所述初始剂量通过注射施用。

36.权利要求18-35的任一项的方法,其中所述初始剂量(i)每14天施用一次;(ii)每21天施用一次;(iii)每28天施用一次,或(iv)每42天施用一次。

37.权利要求18-35的任一项的方法,其中如果:

(a)Runx2水平相比参考群体中的Runx2水平提高;

(b)Alp水平相比参考群体中的Alp水平提高;

(c)Snai1水平相比参考群体中的Snai1水平提高;

(d)phosphosmad2水平相比参考群体中的phosphosmad2水平提高;

(e)Dkk1水平相比参考群体中的Dkk1水平提高;

(f)col1a1水平相比参考群体中的col1a1水平提高;

(g)激活素水平相比参考群体中的激活素水平提高;

(h)BSAP水平相比参考群体中的BSAP水平提高;

(i)CTX水平相比参考群体中的CTX水平提高;

(j)Osterix水平相比参考群体中的Osterix水平提高;

(k)Klotho水平相比参考群体中的Klotho水平降低;

(l)alpha-SMA水平相比参考群体中的alpha-SMA水平降低;

(m)MYOCD水平相比参考群体中的MYOCD水平降低;

(n)Sm22-alpha水平相比参考群体中的Sm22-alpha水平降低;

(o)phosphosmad3水平相比参考群体中的phosphosmad3水平提高;

(p)尿蛋白水平相比参考群体中的尿蛋白水平提高;

(q)ActRIIA水平相比参考群体中的ActRIIA水平降低;和/或

(r)Axin2水平相比参考群体中的Axin2水平降低,

所述ActRII信号转导抑制剂的所述调整的剂量大于所述初始剂量。

38.权利要求37的方法,其中所述调整的剂量大于所述初始剂量约2.5mg、约5mg、约10mg、约15mg、约20mg或约35mg,或大于所述初始剂量约0.05mg/kg、约0.1mg/kg、约0.15mg/kg、约0.25mg/kg、约0.3mg/kg、约0.35mg/kg、约0.4mg/kg或约0.5mg/kg。

39.权利要求37或权利要求38的方法,其中所述调整的剂量比所述初始剂量更频繁地施用。

40.权利要求18-39的任一项的方法,其中所述调整的剂量每5、10、15、20、25、28、30、35

或40天施用。

41.权利要求18-33的任一项的方法，其中如果：

(a)Runx2水平相比参考群体中的Runx2水平降低；

(b)Alp水平相比参考群体中的Alp水平降低；

(c)Snai1水平相比参考群体中的Snai1水平降低；

(d)phosphosmad2水平相比参考群体中的phosphosmad2水平降低；

(e)Dkk1水平相比参考群体中的Dkk1水平降低；

(f)col1a1水平相比参考群体中的col1a1水平降低；

(g)激活素水平相比参考群体中的激活素水平降低；

(h)BSAP水平相比参考群体中的BSAP水平降低；

(i)CTX水平相比参考群体中的CTX水平降低；

(j)Osterix水平相比参考群体中的Osterix水平降低；

(k)Klotho水平相比参考群体中的Klotho水平提高；

(l)alpha-SMA水平相比参考群体中的alpha-SMA水平提高；

(m)Klotho水平相比参考群体中的MYOCD水平提高；

(n)Sm22-alpha水平相比参考群体中的Sm22-alpha水平提高；

(o)phosphosmad3水平相比参考群体中的phosphosmad3水平降低；和/或

(p)尿蛋白水平相比参考群体中的尿蛋白水平降低；

(q)ActRIIA水平相比参考群体中的ActRIIA水平提高；和/或

(r)Axin2水平相比参考群体中的Axin2水平提高，

所述ActRII信号转导抑制剂的所述调整的剂量小于所述初始剂量。

42.权利要求41的方法，其中所述调整的剂量小于所述初始剂量约2.5mg、约5mg、约10mg、约15mg、约20mg或约35mg，或小于所述初始剂量约0.05mg/kg、约0.1mg/kg、约0.15mg/kg、约0.25mg/kg、约0.3mg/kg、约0.35mg/kg、约0.4mg/kg或约0.5mg/kg。

43.权利要求41或42的方法，其中所述调整的剂量比所述初始剂量更不频繁地施用。

44.权利要求41-43的任一项的方法，其中所述调整的剂量每30、35、40、42、50、60、70、80或90天施用。

45.权利要求18-44的任一项的方法，其中所述调整的剂量连续地和/或无限地施用。

46.权利要求18-44的任一项的方法，其中所述第一次测量在治疗的开始之前进行。

47.权利要求18-44的任一项的方法，其中所述第一次测量在治疗的开始之后立即地，或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行。

48.权利要求18-44的任一项的方法，其中所述第二次测量在治疗的开始之后立即地，或在其最多1天、2天、3天、4天、5天、6天或1周、2周、1个月、2个月、3个月、4个月、5个月、6个月、7个月、8个月、9个月、10个月、11个月或12个月之内进行。

49.权利要求37的方法，其中

(a)所述Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的提高的水平分别高于参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的水平约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％；和/

或

(b)所述Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的降低的水平分别低于参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%或100%。

50.权利要求49的方法,其中

(a)所述Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的提高的水平等于或约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%、100%、200%或500%高于参考群体的前10%、前5%、前4%、前3%、前2%或前1%中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的水平;和/或

(b)所述Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的降低的水平等于或约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%或100%低于参考群体的后10%、后5%、后4%、后3%、后2%或后1%中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平。

51.权利要求41的方法,其中

(a)所述Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的提高的水平分别高于参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%、100%、200%或500%;和/或

(b)所述Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的降低的水平分别低于参考群体中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的水平约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%或100%。

52.权利要求51的方法,其中

(a)所述Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的提高的水平等于或约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%、100%、200%或500%高于参考群体的前10%、前5%、前4%、前3%、前2%或前1%中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平;和/或

(b)所述Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的降低的水平等于或约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%或100%低于参考群体的后10%、后5%、后4%、后3%、后2%或后1%中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX和/或Osterix的水平。

53.权利要求1-17的任一项的方法,其中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平分别是Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的蛋白质水平。

54.权利要求18-52的任一项的方法,其中Snail、phosphosmad2、phosphosmad3、尿蛋

白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平分别是Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的蛋白质水平。

55.权利要求53或54的方法，其中所述蛋白质水平通过酶联免疫吸附分析(ELISA)来测定。

56.权利要求55的方法，其中进行所述ELISA使用

(a)Runx2特异性抗体SC-390715(Santa Cruz)来测定Runx2水平；

(b)Alp特异性抗体SC-98652(Santa Cruz)来测定Alp水平；

(c)Snai1特异性抗体sc-393172(Santa Cruz)来测定Snai1水平；

(d)Phosphosmad2特异性抗体sc-101801(Santa Cruz)来测定phosphosmad2水平；

(e)phosphosmad3特异性抗体sc-130218(Santa Cruz)来测定phosphosmad3水平；

(f)Dkk1特异性抗体sc-374574(Santa Cruz)来测定Dkk1水平；

(g)col1a1特异性抗体sc-8784(Santa Cruz)来测定col1a1水平；

(h)激活素-特异性抗体A1594(Sigma Aldrich)来测定激活素水平；

(i)BSAP特异性抗体SC-98652(Santa Cruz)来测定BSAP水平；

(j)CTX-特异性抗体ABIN1173415(Antibodies Online)来测定CTX水平；

(k)Osterix特异性抗体SC-22538(Santa Cruz)来测定Osterix水平；

(l)Klotho特异性抗体SC-22218(Santa Cruz)来测定Klotho水平；

(m)alpha-SMA特异性抗体SC-53142(Santa Cruz)来测定alpha-SMA水平；

(n)MYOCD特异性抗体SC-21561(Santa Cruz)来测定MYOCD水平；和/或

(o)Sm22-alpha特异性抗体SC-271719(Santa Cruz)来测定Sm22-alpha水平

(p)ActRIIA-特异性抗体ab135634(Abcam)来测定ActRIIA水平。

57.权利要求1-17的任一项的方法，其中所述Snai1、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平分别是Snai1、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的mRNA水平。

58.权利要求18-52的任一项的方法，其中所述Snai1、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平分别是Snai1、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的mRNA水平。

59.权利要求57或58的方法，其中所述mRNA水平通过定量逆转录聚合酶链式反应(qRT-PCR)来测定。

60.权利要求59的方法，其中进行QRT-PCR使用

(a)Runx2特异性引物(SEQ ID NO:48和49)来测定Runx2水平；

(b)Alp特异性引物(SEQ ID NO:50和51)来测定Alp水平；

(c)col1a1特异性引物(SEQ ID NO:82和83)来测定col1a1水平；

(d)Snai1特异性引物(SEQ ID No:78和79)来测定Snai1水平；

(e)Dkk1特异性引物(SEQ ID NO:80和81)来测定Dkk1水平；

(f)激活素特异性引物(SEQ ID NO:84和85)来测定激活素水平;

(g)Osterix特异性引物(SEQ ID NO:52和53)来测定Osterix水平;

(h)Klotho特异性引物(SEQ ID NO:54和55)来测定Klotho水平;和/或

(i)Sm22-alpha特异性引物(SEQ ID NO:56和57)来测定Sm22-alpha水平。

61.权利要求1-60的任一项的方法,其中所述水平是在组织中的。

62.权利要求61的方法,其中所述组织是主动脉、血清、骨髓、肝脏或脾脏。

63.权利要求2、3、18或25的任一项的方法,其中所述血管钙化是钙化性动脉粥样硬化、钙化的中层血管病变(也称为Mönckeberg's中层钙化性硬化症)、中层钙化、弹性蛋白沉积、钙化尿毒症性动脉病变,钙化性主动脉瓣膜狭窄或门静脉钙化。

64.权利要求1、19或26的方法,其中所述心血管疾病是与以下相关的疾病:血管钙化例如动脉粥样硬化、高脂血症、骨质疏松症、高血压、炎症、2型糖尿病、终末期肾病、所需截肢、弹性假黄瘤、先天性二尖瓣、风湿性心脏病、门静脉高压症或肝脏疾病。

65.权利要求1、19或26的方法,其中所述心血管疾病继发于慢性肾病。

66.权利要求65的方法,其中所述慢性肾病是3、4或5期慢性肾病。

67.权利要求65的方法,其中所述慢性肾病是慢性肾病矿物质和骨疾病。

68.权利要求1-67的任一项的方法,其中所述ActRII信号转导抑制剂是包含选自以下的氨基酸序列的多肽:

(a)90%相同于SEQ ID NO:2;

(b)95%相同于SEQ ID NO:2;

(c)98%相同于SEQ ID NO:2;

(d)SEQ ID NO:2;

(e)90%相同于SEQ ID NO:3;

(f)95%相同于SEQ ID NO:3;

(g)98%相同于SEQ ID NO:3;

(h)SEQ ID NO:3;

(i)90%相同于SEQ ID NO:6;

(j)95%相同于SEQ ID NO:6;

(k)98%相同于SEQ ID NO:6;

(l)SEQ ID NO:6;

(m)90%相同于SEQ ID NO:7;

(n)95%相同于SEQ ID NO:7;

(o)98%相同于SEQ ID NO:7;

(p)SEQ ID NO:7;

(q)90%相同于SEQ ID NO:12;

(r)95%相同于SEQ ID NO:12;

(s)98%相同于SEQ ID NO:12;

(t)SEQ ID NO:12;

(u)90%相同于SEQ ID NO:17;

(v)95%相同于SEQ ID NO:17;

(w)98％相同于SEQ ID NO:17；

(x)SEQ ID NO:17；

(y)90％相同于SEQ ID NO:20；

(z)95％相同于SEQ ID NO:20；

(aa)98％相同于SEQ ID NO:20；

(bb)SEQ ID NO:20；

(cc)90％相同于SEQ ID NO:21；

(dd)95％相同于SEQ ID NO:21；

(ee)98％相同于SEQ ID NO:21;和

(ff)SEQ ID NO:21。

69.权利要求1-68的任一项的方法，其中所述ActRII信号转导抑制剂是包含SEQ ID NO:7的氨基酸序列的多肽。

70.权利要求1-69的任一项的方法，其中所述ActRII信号转导抑制剂是人源化的融合蛋白，其由ActRIIA的细胞外结构域和人IgG1Fc结构域组成。

71.权利要求1-70的任一项的方法，其中所述对象是人。

72.权利要求1-71的任一项的方法，其中所述参考群体由1、5、10、25、50、75、100、200、250、300、400、500或1000个个体组成。

73.权利要求1-72的任一项的方法，其中所述参考群体由健康人组成。

74.权利要求1-72的任一项的方法，其中所述参考群体由与所述对象相同年龄、体重和/或性别的人组成。

75.权利要求1-72的任一项的方法，其中所述参考群体由没有心血管疾病的人组成。

76.权利要求1-72的任一项的方法，其中所述参考群体由没有血管钙化的人组成。

77.权利要求1-72的任一项的方法，其中所述参考群体由没有肾病的人组成。

78.权利要求1-72的任一项的方法，其中所述参考群体由没有慢性肾病的人组成。

79.权利要求1-72的任一项的方法，其中所述参考群体由没有病理性动脉硬化水平升高的人组成。

80.权利要求1-72的任一项的方法，其中所述参考群体由没有左心室肥大的人组成。

利用ACTRII配体陷阱治疗心血管疾病

[0001] 1.相关申请的交叉引用

[0002] 本申请要求2014年10月9日提交的美国临时专利申请No.62/062,021;2014年11月11日提交的美国临时专利申请No.62/078,321;2015年1月14日提交的美国临时专利申请No.62/103,515;2015年5月27日提交的美国临时专利申请No.62/167,052;和2015年6月2日提交的美国临时专利申请No.62/170,015的优先权;为了所有目的将它们每一篇的全部内容通过引用合并在文本中。

[0003] 2.政府许可权

[0004] 本发明是利用国家卫生研究所颁发的款项编号DK070790和DK089137的政府支持下作出的。政府在本发明中拥有一定权利。

[0005] 3.序列表

[0006] 本申请与文件名为12827_934_228_SeqListing.txt、大小208千字节、2015年10月6日创建的序列表一起提交。为了所有目的,序列表通过完全引用合并在本文中。

4.技术领域

[0007] 本文提供的是治疗和/或预防对象的心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病、和/或与肾病相关和/或由肾病引起的心血管疾病的方法,包括向所述对象施用激活素II型受体信号转导抑制剂(ActRII信号转导抑制剂,例如,激活素配体陷阱(activin ligand trap))。更具体地,本文提供的是选择对象来治疗心血管疾病、血管钙化、动脉硬化水平升高、左心室肥大、与血管钙化相关和/或由血管钙化引起的心血管疾病、与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病、与左心室肥大相关和/或由左心室肥大引起的心血管疾病、和/或与肾病相关和/或由肾病引起的心血管疾病的方法,通过利用一种或更多种生物标记物的水平和/或活性,特别是,snail同源物1(Snail)、phosphosmad2、phosphosmad3、尿蛋白、dickkopf同源物1(Dkk1)、1型胶原蛋白alpha 1(Col1a1)、激活素(例如,游离激活素)、runt相关转录因子2(Runx2)、碱性磷酸酶(Alp)、骨特异性碱性磷酸酶(BSAP)、Osterix、C-末端1型胶原蛋白端肽(CTX)、Klotho、alpha-平滑肌动蛋白(alpha-SMA)、心肌素(myocardin,MYOCD)、axis抑制蛋白质2(Axin2)和/或平滑肌蛋白22-alpha(Sm22-alpha),作为用激活素II型受体(ActRII)信号转导抑制剂治疗的对象对治疗的响应、治疗效果或适当剂量的指示。本文提供的是降低对象的骨吸收的方法,包括向所述对象施用ActRII信号转导抑制剂。更具体地,本文提供的是选择对象来降低骨吸收的方法,通过利用一种或更多种生物标记物的水平和/或活性,特别是Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、Osterix、CTX、Klotho、alpha-SMA、MYOCD和/或Sm22-alpha,作为用ActRII信号转导抑制剂治疗的对象对治疗的响应、治疗效果或适当剂量的指示。

[0008] 5.背景

[0009] 涉及贫血的肾病的常见并发症是血管钙化,在很多情况下其导致心血管疾病。在肾脏对象中,除了肾脏疾病本身相关的死亡之外,动脉粥样硬化和所产生的心血管疾病可

能导致显著的死亡。因而，对于发现和开发用于肾脏对象的心血管疾病的新药物以及治疗和/或预防方法存在着持续的需求。另外，由于肾脏对象经常患有贫血，有益的是用单一的治疗剂来治疗贫血和伴随的心血管疾病，这是当前使用的红细胞生成刺激试剂(ESAs)例如促红细胞生成素所不具有的能力。

[0010] 两种相关的II型受体ActRIIA和ActRIIB已经被鉴定为激活素的II型受体(Mathews and Vale,1991,Cell 65:973-982;Attisano et al.,1992,Cell 68:97-108)。除了激活素之外，ActRIIA和ActRIIB可以与几种其他的TGF-beta家族蛋白，包括BMP7、Nodal、GDF8和GDF11生物化学地相互作用(Yamashita et al.,1995,J.Cell Biol.130:217-226;Lee and McPherron,2001,Proc.Natl.Acad.Sci.98:9306-9311;Yeo and Whitman,2001,Mol.Cell 7:949-957;Oh et al.,2002,Genes Dev.16:2749-54)。ALK4是激活素的主要的I型受体，特别是对于激活素A，ALK-7也可以充当激活素的受体，特别是对于激活素B。

[0011] 激活素配体陷阱，由人源化的融合蛋白组成，所述融合蛋白由激活素IIA型受体(ActRIIA)的细胞外结构域和人IgG1Fc组成(在此称为ActRIIA-hFc或“Sotatercept”;SEQ ID NO:7)，当前在II期临床试验中被评估，用于治疗患有与终末期肾病(ESRD)相关的贫血和骨失调的对象以及患有beta-地中海贫血的那些对象。在健康的绝经后女性中，ActRIIA-hFc也显示了显著地提高血细胞比容(Hct)和血红蛋白(Hgb)以及骨矿物密度。

[0012] 6.概述

[0013] 本文提供的是治疗和/或预防对象的血管钙化的方法，包括向所述对象施用药学上有效剂量的激活素II型受体(ActRII)信号转导抑制剂(例如，激活素配体陷阱)，其中所述对象具有：(a)与参考群体中的Runx2水平相比提高的Runx2水平；(b)与参考群体中的Alp水平相比提高的Alp水平；(c)与参考群体中的Snai1水平相比提高的Snai1水平；(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平；(e)与参考群体中的Dkk1水平相比提高的Dkk1水平；(f)与参考群体中的col1a1水平相比提高的col1a1水平；(g)与参考群体中的激活素水平相比提高的激活素(例如，游离激活素)水平；(h)与参考群体中的BSAP水平相比提高的BSAP水平；(i)与参考群体中的Osterix水平相比提高的Osterix水平；(j)与参考群体中的CTX水平相比提高的CTX水平；(k)与参考群体中的Klotho水平相比降低的Klotho水平；(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平；(m)与参考群体中的MYOCD水平相比降低的MYOCD水平；(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平；(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平；(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平；和/或(q)与参考群体中的ActRIIA水平相比降低的激活素2A型受体(ActRIIA)水平。参见，例如，8.6章节对参考群体的描述。

[0014] 本文提供的是治疗和/或预防对象的心血管疾病的方法，包括向所述对象施用药学上有效剂量的激活素II型受体(ActRII)信号转导抑制剂(例如，激活素配体陷阱)，其中所述对象具有：(a)与参考群体中的Runx2水平相比提高的Runx2水平；(b)与参考群体中的Alp水平相比提高的Alp水平；(c)与参考群体中的Snai1水平相比提高的Snai1水平；(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平；(e)与参考群体中的Dkk1水平相比提高的Dkk1水平；(f)与参考群体中的col1a1水平相比提高的col1a1水平；(g)与参考群体中的激活素水平相比提高的激活素(例如，游离激活素)水平；(h)与参考群体中的

BSAP水平相比提高的BSAP水平;(i)与参考群体中的Osterix水平相比提高的Osterix水平;(j)与参考群体中的CTX水平相比提高的CTX水平;(k)与参考群体中的Klotho水平相比降低的Klotho水平;(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

[0015] 本文提供的是治疗和/或预防对象的心血管疾病的方法,包括向所述对象施用药学上有效剂量的激活素II型受体(ActRII)信号转导抑制剂(例如,激活素配体陷阱),其中所述对象具有:(a)与参考群体中的Runx2水平相比提高的Runx2水平;(b)与参考群体中的Alp水平相比提高的Alp水平;(c)与参考群体中的Snail水平相比提高的Snail水平;(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;(f)与参考群体中的colla1水平相比提高的colla1水平;(g)与参考群体中的激活素水平相比提高的激活素(例如,游离激活素)水平;(h)与参考群体中的BSAP水平相比提高的BSAP水平;(i)与参考群体中的Osterix水平相比提高的Osterix水平;(j)与参考群体中的CTX水平相比提高的CTX水平;(k)与参考群体中的Klotho水平相比降低的Klotho水平;(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平,以及其中所述心血管疾病与血管钙化相关和/或由血管钙化引起。

[0016] 本文提供的是治疗和/或预防对象的心血管疾病的方法,包括向所述对象施用药学上有效剂量的激活素II型受体(ActRII)信号转导抑制剂(例如,激活素配体陷阱),其中所述对象具有:(a)与参考群体中的Runx2水平相比提高的Runx2水平;(b)与参考群体中的Alp水平相比提高的Alp水平;(c)与参考群体中的Snail水平相比提高的Snail水平;(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平;(e)与参考群体中的Dkk1水平相比提高的Dkk1水平;(f)与参考群体中的colla1水平相比提高的colla1水平;(g)与参考群体中的激活素水平相比提高的激活素(例如,游离激活素)水平;(h)与参考群体中的BSAP水平相比提高的BSAP水平;(i)与参考群体中的Osterix水平相比提高的Osterix水平;(j)与参考群体中的CTX水平相比提高的CTX水平;(k)与参考群体中的Klotho水平相比降低的Klotho水平;(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平;(m)与参考群体中的MYOCD水平相比降低的MYOCD水平;(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平;(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平;(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平;和/或(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平,以及其中所述心血管疾病与肾病相关和/或由肾病引起。

[0017] 本文提供的是降低对象的骨吸收的方法,包括向所述对象施用药学上有效剂量的激活素II型受体(ActRII)信号转导抑制剂(例如,激活素配体陷阱),其中所述对象具有:(a)与参考群体中的Runx2水平相比提高的Runx2水平;(b)与参考群体中的Alp水平相比提

高的Alp水平；(c)与参考群体中的Snail水平相比提高的Snail水平；(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平；(e)与参考群体中的Dkk1水平相比提高的Dkk1水平；(f)与参考群体中的colla1水平相比提高的colla1水平；(g)与参考群体中的激活素水平相比提高的激活素(例如，游离激活素)水平；(h)与参考群体中的BSAP水平相比提高的BSAP水平；(i)与参考群体中的Osterix水平相比提高的Osterix水平；(j)与参考群体中的CTX水平相比提高的CTX水平；(k)与参考群体中的Klotho水平相比降低的Klotho水平；(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平；(m)与参考群体中的MYOCD水平相比降低的MYOCD水平；(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平；(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平；(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平；和/或(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

[0018] 本文提供的是治疗和/或预防对象的动脉硬化的方法，包括向所述对象施用药学上有效剂量的激活素II型受体(ActRII)信号转导抑制剂(例如，激活素配体陷阱)，其中所述对象具有：(a)与参考群体中的Runx2水平相比提高的Runx2水平；(b)与参考群体中的Alp水平相比提高的Alp水平；(c)与参考群体中的Snail水平相比提高的Snail水平；(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平；(e)与参考群体中的Dkk1水平相比提高的Dkk1水平；(f)与参考群体中的colla1水平相比提高的colla1水平；(g)与参考群体中的激活素水平相比提高的激活素(例如，游离激活素)水平；(h)与参考群体中的BSAP水平相比提高的BSAP水平；(i)与参考群体中的Osterix水平相比提高的Osterix水平；(j)与参考群体中的CTX水平相比提高的CTX水平；(k)与参考群体中的Klotho水平相比降低的Klotho水平；(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平；(m)与参考群体中的MYOCD水平相比降低的MYOCD水平；(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平；(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平；(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平；和/或(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

[0019] 本文提供的是治疗和/或预防对象的左心室肥大的方法，包括向所述对象施用药学上有效剂量的激活素II型受体(ActRII)信号转导抑制剂(例如，激活素配体陷阱)，其中所述对象具有：(a)与参考群体中的Runx2水平相比提高的Runx2水平；(b)与参考群体中的Alp水平相比提高的Alp水平；(c)与参考群体中的Snail水平相比提高的Snail水平；(d)与参考群体中的phosphosmad2水平相比提高的phosphosmad2水平；(e)与参考群体中的Dkk1水平相比提高的Dkk1水平；(f)与参考群体中的colla1水平相比提高的colla1水平；(g)与参考群体中的激活素水平相比提高的激活素(例如，游离激活素)水平；(h)与参考群体中的BSAP水平相比提高的BSAP水平；(i)与参考群体中的Osterix水平相比提高的Osterix水平；(j)与参考群体中的CTX水平相比提高的CTX水平；(k)与参考群体中的Klotho水平相比降低的Klotho水平；(l)与参考群体中的alpha-SMA水平相比降低的alpha-SMA水平；(m)与参考群体中的MYOCD水平相比降低的MYOCD水平；(n)与参考群体中的Sm22-alpha水平相比降低的Sm22-alpha水平；(o)与参考群体中的phosphosmad3水平相比提高的phosphosmad3水平；(p)与参考群体中的尿蛋白水平相比提高的尿蛋白水平；和/或(q)与参考群体中的ActRIIA水平相比降低的ActRIIA水平。

[0020] 在某些实施方式中，所述药学上有效剂量的所述ActRII信号转导抑制剂是约15mg、约30mg、约45mg、约60mg、约75mg、约90mg或约1g或约0.1mg/kg、约0.13mg/kg、约0.2mg/kg、约0.26mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg。在某些实施方式中，所述药学上有效剂量的ActRII信号转导抑制剂是约0.1mg/kg。在某些实施方式中，所述药学上有效剂量的ActRII信号转导抑制剂是约0.3mg/kg。在某些实施方式中，所述药学上有效剂量的ActRII信号转导抑制剂是约0.5mg/kg。在某些实施方式中，所述药学上有效剂量的ActRII信号转导抑制剂是约0.7mg/kg。

[0021] 在某些实施方式中，所述药学上有效的剂量通过注射来施用。在某些实施方式中，所述药学上有效的剂量(i)每28天一次；或(ii)每42天一次施用。在某些实施方式中，所述药学上有效的剂量每14天施用一次。在某些实施方式中，所述药学上有效的剂量每21天施用一次。在某些实施方式中，所述药学上有效的剂量连续地和/或无限地施用。

[0022] 在某些实施方式中，Snail、phosphosmad2、Dkk1、coll a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的提高的水平分别比参考群体中Snail、phosphosmad2、Dkk1、coll a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterixs水平高约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％。

[0023] 在某些实施方式中，Snail、phosphosmad2、Dkk1、coll a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的提高的水平分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％高于参考群体的前10％、前5％、前4％、前3％、前2％或前1％中Snail、phosphosmad2、Dkk1、coll a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterixs的水平。

[0024] 在某些实施方式中，Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的降低的水平分别比参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平低约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％。

[0025] 在某些实施方式中，Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的降低的水平分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％低于参考群体的后10％、后5％、后4％、后3％、后2％或后1％中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平。

[0026] 本文提供的是治疗和/或预防对象的血管钙化的方法，其中所述方法包括：(a)向所述对象施用初始剂量的ActRII信号转导抑制剂；(b)进行所述对象中Snail、phosphosmad2、Dkk1、coll a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量；(c)一段时间以后，进行所述对象中Snail、phosphosmad2、Dkk1、coll a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量；和(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂。在某些实施方式中，所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化。在某些实施方式

中，所述剂量是如章节8.3.4中描述调整的。

[0027] 本文提供的是治疗和/或预防对象的心血管疾病的方法，其中所述方法包括：(a)向所述对象施用初始剂量的ActRII信号转导抑制剂；(b)进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量；(c)一段时间以后，进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量；和(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂。在某些实施方式中，所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化。在某些实施方式中，所述剂量是如章节8.3.4中描述调整的。

[0028] 本文提供的是治疗和/或预防对象的心血管疾病的方法，其中所述方法包括：(a)向所述对象施用初始剂量的ActRII信号转导抑制剂；(b)进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量；(c)一段时间以后，进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量；和(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂；其中所述心血管疾病与血管钙化相关和/或由血管钙化引起。在某些实施方式中，所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化。在某些实施方式中，所述剂量是如章节8.3.4中描述调整的。

[0029] 本文提供的是治疗和/或预防对象的心血管疾病的方法，其中所述方法包括：(a)向所述对象施用初始剂量的ActRII信号转导抑制剂；(b)进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量；(c)一段时间以后，进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量；和(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂；其中所述心血管疾病与肾病相关和/或由肾病引起。在某些实施方式中，所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化。在某些实施方式中，所述剂量是如章节8.3.4中描述调整的。

[0030] 本文提供的是降低对象的骨吸收的方法，其中所述方法包括：(a)向所述对象施用初始剂量的ActRII信号转导抑制剂；(b)进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量；(c)一段时间以后，进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、

ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂。在某些实施方式中,骨吸收是如章节8.6中评估的。在某些实施方式中,所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化。在某些实施方式中,所述剂量是如章节8.3.4中描述调整的。

[0031] 本文提供的是治疗和/或预防对象的动脉硬化的方法,其中所述方法包括:(a)向所述对象施用初始剂量的ActRII信号转导抑制剂;(b)进行所述对象中Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;(c)一段时间以后,进行所述对象中Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂。在某些实施方式中,动脉硬化的是如章节8.6中评估的。在某些实施方式中,所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化。在某些实施方式中,所述剂量是如章节8.3.4中描述调整的。

[0032] 本文提供的是治疗和/或预防对象的左心室肥大的方法,其中所述方法包括:(a)向所述对象施用初始剂量的ActRII信号转导抑制剂;(b)进行所述对象中Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第一次测量;(c)一段时间以后,进行所述对象中Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平的第二次测量;和(d)向所述对象施用调整的剂量的所述激活素II型受体信号转导抑制剂。在某些实施方式中,所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化。在某些实施方式中,所述剂量是如章节8.3.4中描述调整的。

[0033] 在某些实施方式中,所述初始剂量的所述ActRII信号转导抑制剂是约15mg、约30mg、约45mg、约60mg、约75mg、约90mg或约1g或约0.1mg/kg、约0.13mg/kg、约0.2mg/kg、约0.26mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg。在某些实施方式中,所述初始剂量的所述ActRII信号转导抑制剂是约0.1mg/kg。在某些实施方式中,所述初始剂量的所述ActRII信号转导抑制剂是约0.3mg/kg。在某些实施方式中,所述初始剂量的所述ActRII信号转导抑制剂是约0.5mg/kg。在某些实施方式中,所述初始剂量的所述ActRII信号转导抑制剂是约0.7mg/kg。

[0034] 在某些实施方式中,所述初始剂量是通过注射施用的。在某些实施方式中,所述初始剂量是(i)每28天施用一次;或(ii)每42天施用一次。在某些实施方式中,所述初始剂量是每14天施用一次。在某些实施方式中,所述初始剂量是每21天施用一次。

[0035] 在某些实施方式中,ActRII信号转导抑制剂的所述调整的剂量大于所述初始剂量,如果:(a)与参考群体中Runx2的水平相比Runx2的水平升高;(b)与参考群体中Alp的水平相比Alp的水平升高;(c)与参考群体中Snail的水平相比Snail的水平升高;(d)与参考群

体中phosphosmad2的水平相比phosphosmad2的水平升高；(e)与参考群体中Dkk1的水平相比Dkk1的水平升高；(f)与参考群体中Colla1的水平相比Colla1的水平升高；(g)与参考群体中激活素的水平相比激活素的水平升高；(h)与参考群体中BSAP的水平相比BSAP的水平升高；(i)与参考群体中CTX的水平相比CTX的水平升高；(j)与参考群体中Osterix的水平相比Osterix的水平升高；(k)与参考群体中Klotho的水平相比Klotho的水平降低；(l)与参考群体中alpha-SMA的水平相比alpha-SMA的水平降低；(m)与参考群体中MYOCD的水平相比MYOCD的水平降低；(n)与参考群体中Sm22-alpha的水平相比Sm22-alpha的水平降低；(o)与参考群体中phosphosmad3的水平相比ppospposmad3的水平升高；(p)与参考群体中尿蛋白的水平相比尿蛋白的水平升高；(q)与参考群体中ActRIIA的水平相比ActRIIA的水平降低；和/或(r)与参考群体中Axin2的水平相比Axin2的水平降低。

[0036] 在某些实施方式中，所述调整的剂量是约2.5mg、约5mg、约10mg、约15mg、约20mg或约35mg大于所述初始剂量，或约0.05mg/kg、约0.1mg/kg、约0.15mg/kg、约0.25mg/kg、约0.3mg/kg、约0.35mg/kg、约0.4mg/kg或约0.5mg/kg大于所述初始剂量。在某些实施方式中，所述调整的剂量比所述初始剂量更频繁地施用。在某些实施方式中，所述调整的剂量每5、10、15、20、25、28、30、35或40天施用。

[0037] 在某些实施方式中，ActRII信号转导抑制剂的所述调整的剂量小于所述初始剂量，如果：(a)与参考群体中Runx2的水平相比Runx2的水平降低；(b)与参考群体中Alp的水平相比Alp的水平降低；(c)与参考群体中BSAP的水平相比BSAP的水平降低；(d)与参考群体中Snai1的水平相比Snai1的水平降低；(e)与参考群体中phosphosmad2的水平相比phosphosmad2的水平降低；(f)与参考群体中Dkk1的水平相比Dkk1的水平降低；(g)与参考群体中colla1的水平相比colla1的水平降低；(h)与参考群体中激活素(例如，游离激活素)的水平相比激活素(例如，游离激活素)的水平降低；(i)与参考群体中CTX的水平相比CTX的水平降低；(j)与参考群体中Osterix的水平相比Osterix的水平降低；(k)与参考群体中Klotho的水平相比Klotho的水平升高；(l)与参考群体中alpha-SMA的水平相比alpha-SMA的水平升高；(m)与参考群体中MYOCD的水平相比MYOCD的水平升高；(n)与参考群体中Sm22-alpha的水平相比Sm22-alpha的水平升高；(o)与参考群体中phosphosmad3的水平相比ppospposmad3的水平降低；(p)与参考群体中尿蛋白的水平相比尿蛋白的水平降低；(q)与参考群体中ActRIIA的水平相比ActRIIA的水平升高；和/或(r)与参考群体中Axin2的水平相比Axin2的水平升高。

[0038] 在某些实施方式中，所述调整的剂量是约2.5mg、约5mg、约10mg、约15mg、约20mg或约35mg小于所述初始剂量，或约0.05mg/kg、约0.1mg/kg、约0.15mg/kg、约0.25mg/kg、约0.3mg/kg、约0.35mg/kg、约0.4mg/kg或约0.5mg/kg小于所述初始剂量。在某些实施方式中，所述调整的剂量比所述初始剂量较少频繁地施用。在某些实施方式中，所述调整的剂量每30、35、40、42、50、60、70、80或90天施用。在某些实施方式中，所述调整的剂量连续地和/或无限地施用。

[0039] 在某些实施方式中，所述第一次测量在治疗的开始之前进行。在某些实施方式中，所述第一次测量在治疗的开始之后立即进行，或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或2个月之内进行。在某些实施方式中，所述第二次测量在治疗开始后立即进行，或在其最多1天、2天、3天、4天、5天、6天或1周、2周、1个月、2个月、3个月、4个月、5个

月、6个月、7个月、8个月、9个月、10个月、11个月或12个月之内进行。

[0040] 在某些实施方式中，(a)Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的升高的水平分别高于参考群体中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的水平约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％；和/或(b)Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的降低的水平分别低于参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％。

[0041] 在某些实施方式中，(a)Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的升高的水平分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％高于参考群体中的前10％、前5％、前4％、前3％、前2％或前1％中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的水平；(b)Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的降低的水平分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％低于参考群体中的后10％、后5％、后4％、后3％、后2％或后1％中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平。

[0042] 在某些实施方式中，(a)Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的升高的水平分别高于参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％，和/或(b)Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的降低的水平分别低于参考群体Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的水平约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％。

[0043] 在某些实施方式中，(a)Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的升高的水平分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％高于参考群体的前10％、前5％、前4％、前3％、前2％或前1％中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平；和/或(b)Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的降低的水平分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％低于参考群体的后10％、后5％、后4％、后3％、后2％或后1％中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的水平。

[0044] 在某些实施方式中，Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平分别是Snai1、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、

phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的蛋白质水平。在某些实施方式中，所述蛋白质水平通过酶联免疫吸附分析(ELISA)来测定。在某些实施方式中，进行ELISA，用(a)Runx2-特异性抗体SC-390715(Santa Cruz)来测定Runx2水平；(b)用Alp-特异性抗体SC-98652(Santa Cruz)来测定Alp水平；(c)Snail-特异性抗体sc-393172(Santa Cruz)来测定Snail水平；(d)用phosphosmad2-特异性抗体sc-101801(Santa Cruz)来测定phosphosmad2水平；(e)用Dkk1-特异性抗体sc-374574(Santa Cruz)来测定Dkk1水平；(f)用Colla1-特异性抗体sc-8784(Santa Cruz)来测定colla1水平；(g)用激活素-特异性抗体A1594(Sigma Aldrich)来测定激活素水平；(h)用BSAP-特异性抗体SC-98652(Santa Cruz)来测定BSAP水平；(i)用CTX-特异性抗体ABIN1173415(Antibodies Online)来测定CTX水平；(j)用Osterix-特异性抗体SC-22538(Santa Cruz)来测定Osterix水平；(k)用Klotho-特异性抗体SC-22218(Santa Cruz)来测定Klotho水平；(l)用alpha-SMA-特异性抗体SC-53142(Santa Cruz)来测定alpha-SMA水平；(m)用MYOCD-特异性抗体SC-21561(Santa Cruz)来测定MYOCD水平；(n)用Sm22-alpha-特异性抗体SC-271719(Santa Cruz)来测定Sm22-alpha水平；(o)用phosphosmad3-特异性抗体sc-11769(Santa Cruz)来测定phosphosmad3水平；和/或(p)用ActRIIA-特异性抗体ab 135634(Abcam)来测定ActRIIA水平。

[0045] 在某些实施方式中，Snail、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的平分别是Snail、Dkk1、colla1、激活素、Runx2,Alp,BSAP,CTX,Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的mRNA水平。在某些实施方式中，所述mRNA水平通过定量逆转录聚合酶链式反应(qRT-PCR)来测定。在某些实施方式中，进行qRT-PCR，用(a)Runx2-特异性引物(SEQ ID NO:48和49)来测定Runx2水平；(b)用Alp-特异性引物(SEQ ID NO:50和51)来测定Alp水平；(c)用Snail-特异性引物(SEQ ID NO:78和79)来测定Snail水平；(d)用Dkk1-特异性引物(SEQ ID NO:80和81)来测定Dkk1水平；(e)用colla1-特异性引物(SEQ ID NO:82和83)来测定colla1水平；(f)用激活素-特异性引物(SEQ ID NO:84和85)来测定激活素水平；(g)用Osterix-特异性引物(SEQ ID NO:52和53)来测定Osterix水平；(h)用Klotho-特异性引物(SEQ ID NO:54和55)来侧Klotho水平；和/或(i)用Sm22-alpha-特异性引物(SEQ ID NO:56和57)来测定Sm22-alpha水平。

[0046] 在某些实施方式中，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2、ActRIIA、Axin2和/或alpha-SMA水平是组织中的。在某些实施方式中，所述组织是主动脉。在某些实施方式中，所述组织是肾脏。在某些实施方式中，所述组织是骨。在某些实施方式中，所述组织是血清。在优选的实施方式中，Runx2、Dkk1、Alp、osterix、sm22-alpha、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2水平和/或活性是在主动脉中的。在优选的实施方式中，phosphosmad3、尿蛋白、phosphosmad2、ActRIIA、Axin2和colla1水平和/或活性是在肾脏中的。在优选的实施方式中，所述激活素水平和/或活性是在血清中的。

[0047] 在某些实施方式中，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、ActRIIA、Axin2和/或

alpha-SMA水平是主动脉中的。

[0048] 在某些实施方案中，所述血管钙化是钙化性动脉粥样硬化、钙化的中层血管病变(也称为Mönckeberg's中层钙化性硬化症)、中层钙化、弹性蛋白沉积、钙化尿毒症性动脉病变，钙化性主动脉瓣膜狭窄或门静脉钙化。

[0049] 在某些实施方式中，所述心血管疾病是与以下相关的疾病：血管钙化例如动脉粥样硬化、高脂血症、骨质疏松症、高血压、炎症、2型糖尿病、终末期肾病、所需截肢、弹性假黄瘤、先天性二尖瓣、风湿性心脏病、门静脉高压症或肝脏疾病。

[0050] 在某些实施方式中，所述心血管疾病是继发于慢性肾病的。在某些实施方式中，所述慢性肾病是3、4或5期慢性肾病。在某些实施方式中，所述慢性肾病是慢性肾病矿物质和骨骼疾病。

[0051] 在某些实施方式中，所述对象患有高周转性骨病，例如，高周转性肾性骨营养不良(ROD)。

[0052] 本文还提供的是一种治疗高周转性骨病，例如，高周转性ROD的方法，包括向对象施用药学上有效量的ActRII信号转导抑制剂。

[0053] 在某些实施方式中，所述ActRII信号转导抑制剂是包含选自以下构成的组的氨基酸序列的多肽：(a)90％相同于SEQ ID NO:2；(b)95％相同于SEQ ID NO:2；(c)98％相同于SEQ ID NO:2；(d)SEQ ID NO:2；(e)90％相同于SEQ ID NO:3；(f)95％相同于SEQ ID NO:3；(g)98％相同于SEQ ID NO:3；(h)SEQ ID NO:3；(i)90％相同于SEQ ID NO:6；(j)95％相同于SEQ ID NO:6；(k)98％相同于SEQ ID NO:6；(l)SEQ ID NO:6；(m)90％相同于SEQ ID NO:7；(n)95％相同于SEQ ID NO:7；(o)98％相同于SEQ ID NO:7；(p)SEQ ID NO:7；(q)90％相同于SEQ ID NO:12；(r)95％相同于SEQ ID NO:12；(s)98％相同于SEQ ID NO:12；(t)SEQ ID NO:12；(u)90％相同于SEQ ID NO:17；(v)95％相同于SEQ ID NO:17；(w)98％相同于SEQ ID NO:17；(x)SEQ ID NO:17；(y)90％相同于SEQ ID NO:20；(z)95％相同于SEQ ID NO:20；(aa)98％相同于SEQ ID NO:20；(bb)SEQ ID NO:20；(cc)90％相同于SEQ ID NO:21；(dd)95％相同于SEQ ID NO:21；(ee)98％相同于SEQ ID NO:21；以及(ff)SEQ ID NO:21。在某些实施方式中，所述ActRII信号转导抑制剂是包含SEQ ID NO:7的氨基酸序列的多肽。在某些实施方式中，所述ActRII信号转导抑制剂是由ActRIIA的细胞外结构域和人IgG1Fc结构域组成的人源化的融合蛋白。

[0054] 在某些实施方式中，所述ActRII信号转导抑制剂是Sotatercept(SEQ ID NO:7；参见，例如，章节9)。Sotatercept是一种激活素配体陷阱，其在本文描述的方法中是有用的(参见章节8)。

[0055] 在某些实施方式中，所述对象是人类。

[0056] 7.附图简述

[0057] 附图1A描绘了与野生型和假(sham)模型相比在肾病的间质性纤维化模型(CKD-3)中提高的激活素-A水平(pg/ml)。附图1B描绘了与野生型(WT)相比在肾病的AIports模型(AIport200d)中提高的激活素-A水平(pg/ml)以及在用BMP-7治疗时提高的激活素-A水平。

[0058] 附图2描绘了与假的相比在CKD-3模型(CKD-3V)的肾脏和主动脉中提高的激活素A(抑制素beta)mRNA水平。用mActRIIA-Fc治疗降低了CKD-3模型中的激活素A mRNA(mActRIIA-Fc)。

[0059] 附图3A描绘了野生型小鼠主动脉中的ACTRII水平。附图3B描绘了在假治疗的小鼠主动脉中的ACTRII水平。附图3C描绘了与附图3A和/或附图3B相比在CKD-3模型的小鼠主动脉中提高的ACTRII水平。附图3D描绘了与附图3C相比在mActRIIA-Fc治疗的CKD-3模型的小鼠主动脉中降低的ACTRII水平。

[0060] 附图4A描绘了在有(CKD3+ActRII-Fc)和没有(CKD3+v)ActRII信号转导抑制剂治疗时慢性肾病小鼠模型中Runx2的mRNA水平。附图4B描绘了在有(CKD3+ActRII-Fc)和没有(CKD3+v)ActRII信号转导抑制剂治疗时慢性肾病小鼠模型中Alp的mRNA水平。附图4C描绘了在有(CKD3+ActRII-Fc)和没有(CKD3+v)ActRII信号转导抑制剂治疗时慢性肾病小鼠模型中Klotho的mRNA水平。附图4D描绘了在有(CKD3+ActRII-Fc)和没有(CKD3+v)ActRII信号转导抑制剂治疗时慢性肾病小鼠模型中心肌素(MYOCD)的mRNA水平。附图4E描绘了在有(CKD3+ActRII-Fc)和没有(CKD3+v)ActRII信号转导抑制剂治疗时慢性肾病小鼠模型中Sm22-alpha(SM22α)的mRNA水平。

[0061] 附图4F描绘了与用单独的溶媒(CKD3+vehicle)治疗或伪治疗的CKD3相比,在用ActRII信号转导抑制剂(CKD3+ActRII-Fc)治疗的CKD-3模型中肌动蛋白alpha-平滑肌、Runx2、Klotho、MYOCD和alpha-微管蛋白的蛋白质水平。

[0062] 附图5A描绘了在用溶媒(veh.Rx)治疗时CKD-3诱导的血管钙化。附图5B描绘了用ActRII信号转导抑制剂(mActRIIA-Fc Rx)治疗时降低的CKD-3诱导的血管钙化。

[0063] 附图6A描绘了与野生型(WT)、假的、用溶媒治疗的CKD-3模型(CKD-3 Veh)相比,钙水平的CKD-3诱导的提高(CKD-3 22wk)。用mActRIIA-Fc治疗降低了CKD-3诱导的钙积累(mActRIIA-Fc)。附图6B描绘了用ActRII信号转导抑制剂(mActRIIA-Fc)治疗的野生型小鼠(WT)、假治疗的小鼠(sham)、CKD-3小鼠模型(CKD-3)或小鼠模型CKD-3的骨量。附图6C描绘了用ActRII信号转导抑制剂(mActRIIA-Fc)治疗的野生型小鼠(WT)、假治疗的小鼠(sham)、CKD-3小鼠模型(CKD-3)或小鼠模型CKD-3的破骨细胞数目。附图6D描绘了用ActRII信号转导抑制剂(mActRIIA-Fc)治疗的野生型小鼠(WT)、假治疗的小鼠(sham)、CKD-3小鼠模型(CKD-3)或小鼠模型CKD-3的破骨细胞表面。

[0064] 附图7A描绘了在GNZ小鼠(Stoller et al,Genesis,2008)培育成内皮特异性Tie2-Cre(也称为“Tek-Cre”)小鼠中进行的细胞谱系跟踪的策略。附图7B描绘了假的小鼠中的内皮细胞谱系跟踪。附图7C描绘了CKD小鼠中的内皮细胞谱系跟踪。附图7D描绘了CKD小鼠中的内皮细胞谱系跟踪。

[0065] 附图8描绘了对象分布。注意:“•”表示对象在基线和第225天有匹配的QCT测量。“*”表示接受安慰剂的对象具有提高的血清促红细胞生成素水平,表明方案之外的促红细胞生成素施用。“†”表示方案违背。“‡”表示对象在第29天满足了血压升高的停止规则指标;研究治疗被停止,在第26天实施挽回治疗,继续跟踪。在基线时,根据不完备的评估,对象随机分配不合格的血压。

[0066] 附图9A描绘了在每个治疗类别中具有大于2%的股骨颈部皮层骨提高的对象百分比。附图9B描绘了在每个治疗组中有腰椎小梁骨质量的任何提高或降低的对象百分比。

[0067] 附图10A描绘了每个治疗类别中总Agatston得分改变的对象的百分比。附图10B描绘了每个治疗类别中平方根转化的总体积得分改变的对象的百分比。

[0068] 附图11描绘了安慰剂(PBO)治疗的对象或用剂量0.3mg/kg(0.3)或0.5mg/kg(0.5)

的ActRII信号转导抑制剂治疗的对象的骨吸收生物标记物(CTX)变化的百分比。

[0069] 附图12A表明用mActRIIA-Fc(CKD-3 R)以10mg/kg每周两次皮下地治疗的CKD-3小鼠对菊糖清除没有影响。附图12B表明用mActRIIA-Fc(CKD-3 R)以10mg/kg每周两次皮下地治疗的CKD-3小鼠对BUN没有影响。附图12A和附图12B利用了带有ablative CKD的血管钙化模型,如章节9.4.1.1中描述的,来产生与称为CKD-3的人类CKD 3期类似的降低的肾脏功能(溶媒治疗的为CKD-3 V),通过菊糖清除和BUN来估计,与WT C57B6小鼠(WT)相比具有GFR的70%降低。附图12C表明200天龄的Col4α5缺陷小鼠(Alport)具有与人类3-4期CKD相当的菊糖清除降低。*p<0.05。附图12D表明200天龄的Col4α5缺陷小鼠(Alport)具有与人类3-4期CDK相当的BUN提高。*p<0.05。

[0070] 附图13表明CKD提高循环中的激活素以及肾脏和主动脉的ActRIIA表达。“WT”=野生型;“sham”=假治疗的小鼠;“CKD-3 V”=用溶媒治疗的小鼠;“CKD-3 R”=用mActRIIA-Fc治疗的小鼠。附图13A描绘了在如章节9.4.1.1中描述的ldlr-/-高脂肪饲喂的CKD-3小鼠模型中通过CKD的循环激活素-A诱导。附图13B描绘了在如章节9.4.1.1中描述的Alport's综合征小鼠中CKD对循环激活素-A的诱导。附图13C表明小鼠肾脏和主动脉中的抑制素betaA表达(激活素-A由抑制素betaA基因的同二聚体形成)。附图13D表明肾脏匀浆中抑制素betaA的蛋白水平。1,2和3是指单个样品。附图13E描绘了主动脉ActRIIA的免疫组织化学。在野生型(WT)和假操作的ldlr-/-高脂肪饲喂小鼠(Sham)的主动脉中检测到ActRIIA表达,其是在用溶媒(CKD-3 V)或mActRIIA-Fc(CKD-3 mActRIIA-Fc)治疗的CKD-3小鼠中刺激出的。比例尺是20μm。附图13F表明,与WT和sham小鼠相比,在CKD-3小鼠(用溶媒治疗的,CKD-3 V;或用mActRIIA-Fc治疗的,CKD-3 R)中循环卵泡抑素水平没有改变。*p<0.05,***p<0.005。附图13G表明,与WT和sham小鼠相比,在CKD-3小鼠(用溶媒治疗的,CKD-3V;或用mActRIIA-Fc治疗的,CKD-3 R)中循环卵泡抑素样3(Fstl3)水平没有改变。*p<0.05,***p<0.005。

[0071] 附图14表明CKD刺激的内皮向间充质转变。附图14A描绘了小鼠繁育策略的图表。ROSA26GNZ敲入小鼠(GNZ小鼠)具有带终止密码子的序列,其防止核定位的GFP-LacZ报告物的表达(GNZ)。当与在内皮细胞中表达Cre重组酶(Cre)的Tek-Cre小鼠繁育时,Cre重组酶(Cre)删除了侧翼有loxP的序列,内皮来源的所有细胞产生GNZ报告物蛋白。附图14B到附图14D展现了使用DAPI核染色,对于内皮细胞标志物CD31和GFP,Tek-Cre/GNZ小鼠主动脉的双倍免疫染色。箭头指向双重染色的内皮细胞。在假操作的小鼠中(附图14B),仅内皮细胞显示了核和细胞质的GFP染色。在CKD3小鼠中,(附图14C和附图14D)除了内皮细胞之外,主动脉中层和外膜的细胞显现了GFP染色(箭头)。比例尺是20μm。附图14E描绘了与EnMT相关的转录因子Snai 1(Medici,D.,et al.,2008.Molecular Biology of the Cell 19:4875-4887.)的主动脉mRNA水平,相比野生型(WT)小鼠,在75天龄的Alport's小鼠中提高。alpha微管蛋白充当加载对照。1和2是指单独的样品。

[0072] 附图15描绘了在有CKD-3的ldlr-/-高脂肪饲喂小鼠中mActRIIA-Fc对血管钙化的作用。附图15A描绘了来自溶媒-(CKD-3 V)和mActRIIA-Fc治疗的(CKD-3 mActRIIA-Fc)CKD-3小鼠的近端主动脉动脉粥样硬化斑块的茜素红染色的切片。附图15B描绘了小鼠的组中的主动脉钙水平:野生型(wt);假操作的ldlr-/-高脂肪饲喂(sham);在治疗的建立之时,在22周安乐死的CKD-3(CKD-3);从22到28周用溶媒治疗的CKD-3(CKD-3V);从22到28周用

mActRIIA-Fc、10mg/kg每周两次皮下治疗的CKD-3(CKD-3 R)。方框代表中值(框中的线条)，四分位数范围从25th到75th百分位。误差棒代表四分位数范围的1.5倍。各组用ANOVA Holm-Sidak方法进行比较，进行多重比较，p<0.05为显著差异水平。*p<0.02，每个组的n为8-12。

[0073] 附图16表明降低的ActRIIA信号转导对CKD刺激的心脏病的作用。附图16A表明ldlr-/-高脂肪饲喂小鼠中的CKD-3提高了心脏重量，与假治疗的小鼠相比，这在mActRIIA-Fc治疗的组中是反转的。CKD-3-L是指用碳酸镧治疗的CKD-3小鼠。附图16B表明产生了主要在左心室的心脏肥大(中间)，与sham小鼠(左侧)相比，这在mActRIIA-Fc治疗的组(右侧)中被防止。三色染色，比例尺1mm。附图16C描绘了在更高放大率下的左室性肌细胞的三色染色，没有展现心脏纤维化的证据。比例尺20μm。

[0074] 附图17展现了ldlr-/-高脂肪饲喂的CKD-3小鼠中CKD-3对血管硬化的作用，以及Dkk1中和作用的缺乏。附图17A描绘了左颈总动脉的如早先的报道(Wagenseil,J.E.,et al.,2009.Circulation Research 104:1217-1224;Wagenseil,J.E.,et al.,2005.AJP-Heart and Circulatory Physiology289:H1209-H1217.)进行的压力-直径关系。附图17B描绘了来自野生型(WT-28周)、假操作的(Sham-28周)、CKD-3(28周)和用Dkk1单克隆抗体治疗的CKD-3(Dkk1mab-28周)小鼠的升主动脉。对于附图17，黑色代表WT或假操作的小鼠，而空心或灰色代表CKD-3小鼠。数值以平均值±标准偏差来呈现。对于颈动脉，N＝5-6只小鼠，升主动脉为3-6只。

[0075] 附图18展现了肾脏和主动脉中的ActRIIA信号转导。附图18A描绘了来自假治疗的、CKD-3溶媒-(CKD-3 V)和mActRIIA-Fc治疗的(CKD-3R)小鼠的肾脏(左侧)和主动脉(右侧)的组织匀浆通过westerns测定的激活素信号转导。在肾脏组织匀浆中而不是在主动脉组织匀浆中激活素水平提高，Alk4(AcvR1B)和Alk2(AcvR1)1型受体存在于肾脏组织匀浆，而CKD-3不提高Alk4磷酸化。Alk4和Alk1(AcvRL1)存在于主动脉组织匀浆中。CKD-3提高了肾脏中而非主动脉中的smad2/3磷酸化，并提高了肾脏Col1A1水平。mActRIIA-Fcz治疗降低了肾脏phosphosmad2/3和Col1A1水平。在主动脉中，CKD-3不仅对phosphosmad2/3水平没有影响，对phosphoErk 1/2水平也没有影响。Runx2水平通过CKD-3提高，而通过mActRIIA-Fc归一化。附图18B描绘了CKD-3小鼠的肾脏中Klotho mRNA表达的降低，以及通过mActRIIA-Fc治疗对它的纠正。附图18C描绘了来自假治疗的、CKD-3溶媒和mActRIIA-Fc治疗的小鼠(左侧)的肾脏和主动脉组织匀浆的westerns中通过Dkk1蛋白表达来标记的Wnt信号转导，以及CKD-3和mActRIIA-Fc对血浆Dkk1水平(右侧)的影响。

[0076] 附图19描绘了患有3期CKD的患者(n＝30)对比健康供体(n＝10)的血浆激活素-A水平。通过使用商业的ELISA试剂盒(R&D Systems,Minneapolis,MN)一式两份测量样品来产生数据。分析的变异系数是2.7％。***p<0.002学生t检验。

[0077] 附图20描绘了野生型小鼠(WT)、假治疗的小鼠(sham)、CKD-3小鼠(CKD-3 V)或用mActRIIA-Fc治疗的CKD-3小鼠(CKD-3 R)中FGF-23的水平。

[0078] 附图21A描绘了野生型小鼠(WT)、假操作的小鼠(Sham)、用溶媒治疗的CKD-3小鼠(CKD-3 V)或用mActRIIA-Fc治疗的CKD-3小鼠(CKD-3 R)中循环激活素A的水平。*表示p<0.005。附图21B描绘了假操作的小鼠(Sham)、用溶媒治疗的CKD-3小鼠(CKD-3 V)或用mActRIIA-Fc治疗的CKD-3小鼠(CKD-3 R)的成骨细胞数量/骨骼周长数/100mm。CKD-3 V vs.CKD-3的p<0.05。附图21C描绘了假操作的小鼠(Sham)、用溶媒治疗的CKD-3小鼠(CKD-3

V)或用mActRIIA-Fc治疗的CKD-3小鼠(CKD-3 R)的成骨细胞表面/骨表面的百分比。CKD-3 V vs.CKD-3的p<0.05。附图21D描绘了假操作的小鼠(Sham)、用溶媒治疗的CKD-3小鼠(CKD-3 V)或用mActRIIA-Fc治疗的CKD-3小鼠(CKD-3 R)的骨形成率/成骨细胞。CKD-3 V vs.CKD-3的p<0.05。

[0079] 附图22A描绘了在以标明的剂量用安慰剂或mActRIIA-Fc治疗时,在研究的过程中的对象分布。y轴的上的每个数字代表单独的对象。附图22B描绘了在以标明的剂量用安慰剂或mActRIIA-Fc治疗时,在对象的腹部主动脉的总Agatston得分上有<15%的发展的对象比例。附图22C描绘了在以标明的剂量用安慰剂或mActRIIA-Fc治疗时,在股骨颈部皮层BMD方面有>2%提高的对象的比例。

[0080] 附图23A描绘了ActRIIA的细胞外结构域与IgG1的Fc结构域的小鼠融合蛋白(mActRIIA-Fc)的示意图。附图23B描绘了在ldlr-/-高脂肪饲喂小鼠中mActRIIA-Fc对CKD-MBD的影响的实验设计。从12周(wks)龄开始,四个组中的小鼠饲喂高脂肪饮食。在12周进行假手术(SO)或电灼皮层损伤(EC)。在14周,进行SO或对侧肾切除(NX)。在生命的22周,研究10mg/kg皮下每周两次的溶媒治疗或mActRIIA-Fc。WT野生型小鼠食物饮食为正常参考水平。Sham,假操作的ldlr-/-高脂肪饲喂小鼠;CKD-3,在治疗开始时在22周研究以建立血管钙水平的CKD-3ldlr-/-高脂肪饲喂小鼠;CKD-3 V,溶媒治疗的CKD-3ldlr-/-高脂肪饲喂小鼠;CKD-3R,mActRIIA-Fc治疗的ldlr-/-高脂肪饲喂小鼠;WT、Sham、CKD-3 V和CKD-3 R小鼠在28周龄处死。

[0081] 附图24A描绘了通过westerns对主动脉组织匀浆中的ActRIIA测定的、小鼠主动脉中ActRIIA的表达。附图24B描绘了附图24A的免疫印迹定量。对于定量,n=4,**p<0.01。附图24C描绘了sham小鼠的主动脉中ActRIIA的免疫荧光检测。附图24D描绘了CKD-3小鼠的主动脉中ActRIIA的免疫荧光检测。ActRIIA在主动脉VSMC中表达,但在内皮细胞中没有检出。与sham相比,在CKD中VSMCActRIIA水平保持为可检测的。CD31(箭头)用作内皮细胞标志物。核用DAPI染色。比例尺20μm。

[0082] 附图25A显示了CKD-3引起成骨细胞蛋白质(Runx2和碱性磷酸酶(Alpl))的提高的mRNA表达,以及降低水平的主动脉平滑肌细胞22α(Tagln)这些全部被mActRIIA-Fc的治疗反转。主动脉的心肌素(Myocd)水平被CKD降低,但是不受mActRIIA-Fc影响。附图25B描绘了主动脉组织匀浆中蛋白质的westerns。附图25C描绘了附图25B的westerns的免疫印迹定量。CKD引起主动脉的α-平滑肌细胞肌动蛋白的水平降低,以及成骨细胞的Runx2的水平提高,其被mActRIIA-Fc的治疗反转,但是心肌素水平没有改变。对于定量,n=4,**p<0.01。

[0083] 附图26A描绘了在患有CKD-3的ldlr-/-高脂肪饲喂小鼠中CKD和ActRIIA配体陷阱对主动脉钙化的影响,显示了用来自溶媒的茜素红对近端主动脉动脉粥样硬化斑块的染色切片。附图26B描绘了在患有CKD-3并用mActRIIA-Fc治疗的ldlr-/-高脂肪饲喂小鼠中CKD和ActRIIA配体陷阱对主动脉钙化的影响,如茜素红染色的近端主动脉动脉粥样硬化斑块所示。箭头表示内膜中的钙沉积(i);m-中层。比例尺100μm。附图26C描绘了小鼠的组中的主动脉钙水平:野生型(WT);假操作的ldlr-/-高脂肪饲喂(Sham);在治疗的建立之时,在22周安乐死的CKD-3(CKD-3);从22到28周用溶媒治疗的CKD-3(CKD-3 V);从22到28周用mActRIIA-Fc、10mg/kg每周两次皮下治疗的CKD-3(CKD-3 R)。方框代表中值(框中的线条),四分位数范围从25th到75th百分位。误差棒代表四分位数范围的1.5倍。1各组用ANOVA

Holm-Sidak方法进行比较;进行多重比较;p<0.05作为显着差异水平。*p<0.02;每个组的n为8-12。

[0084] 附图27A描绘了来自假治疗的、CKD-3溶媒和CKD-3 mActRIIA-Fc治疗的小鼠的主动脉组织匀浆的westerns的ActRIIA信号转导分析。组织匀浆的免疫印迹来自每个组中动物的两条主动脉。在来自CKD-3小鼠的主动脉组织匀浆中ActRIIA和激活素(抑制素β-A)水平降低。Alk4(AcvR1B)和Alk1(AcvRL1)1型受体存在于主动脉组织匀浆中。CKD-3降低了主动脉中的smad2/2磷酸化,其被mActRIIA-Fc治疗提高。CKD-3降低了磷酸-Erk1/2水平。Runx2水平通过CKD-3提高,而通过mActRIIA-Fc归一化。附图27B描绘了p-Smad2/3免疫印迹定量,n=4,**p<0.01。

[0085] 附图28A描绘了野生型小鼠的主动脉中beta-连环蛋白(catenin)表达的免疫荧光显微术。附图28B描绘了CKD小鼠的主动脉中beta-连环蛋白表达的免疫荧光显微术。血管平滑肌细胞中没有beta-连环蛋白的免疫荧光。来自CKD小鼠的主动脉的内皮中有beta-连环蛋白表达。箭头,内皮细胞中beta-连环蛋白和CD31的共同定位。比例尺20μm。附图28C描绘了主动脉的Axin2mRNA表达水平。附图28D描绘了来自sham、CKD-3 V和CKD-3 R治疗的小鼠的主动脉组织匀浆的westerns中,通过Dkk1蛋白表达来标记的Wnt信号转导的分析,以及免疫印迹定量,n=4。附图28E描绘了CKD-3V和mActRIIA-Fc对血浆Dkk1水平的影响。*p<0.05,**p<0.01。

[0086] 附图29A描绘了mActRIIA-Fc治疗对肾脏klotho mRNA水平的影响,表明CKD-3降低肾脏组织匀浆中的klotho基因表达水平,相比CKD-3V,mActRIIA-Fc治疗显著地提高它们。*p<0.05,**p<0.01,***p<0.005。附图29B描绘了来自假治疗的、CKD-3溶媒和CKD-3 mActRIIA-Fc治疗的小鼠的肾脏组织匀浆的westerns的ActRIIA信号转导分析。所有的免疫印迹是来自3或4个肾脏的组织匀浆的表示。ActRIIA不受CKD-3或mActRIIA-Fc的影响。激活素A(抑制素β-A)水平在CKD-3小鼠的肾脏组织匀浆中提高(定量在附图30A-B中显示),被mActRIIA-Fc降低。Alk4(AcvR1B)和Alk2(AcvR1)1型受体存在于肾脏组织匀浆中,但是CKD-3V或CKD-3 R不显著影响Alk4的磷酸化。CKD-3提高了肾脏的smad2/3磷酸化,mActRIIA-Fc治疗降低了肾脏的磷酸smad2/3。附图29C描绘了附图29B的免疫印迹定量),*p<0.01。

[0087] 附图30A描绘了来自CKD-3V mActRIIA-Fc治疗的小鼠的肾脏切片的三色染色。附图30B描绘了来自CKD-3V mActRIIA-Fc治疗的小鼠的肾脏切片的三色染色。附图30C描绘了来自CKD-3V mActRIIA-Fc治疗的小鼠的肾脏切片的三色染色。附图30D描绘了来自CKD-3mActRIIA-Fc治疗的小鼠的肾脏切片的三色染色。间质性纤维化的区域通过箭头标出。CKD-3mActRIIA-Fc治疗的小鼠的肾脏具有降低的间质性纤维化。比例尺50μm。整个肾脏冠状面的标记参见附图35,从其中采集了显微照片的切片。附图30E描绘了mActRIIA-Fc对尿蛋白的影响。在CKD-3V小鼠中有显著的蛋白尿,这通过mActRIIA-Fc治疗降低了,*p<0.05。

[0088] 附图31A描绘了在动脉粥样硬化的ldlr-/-高脂肪饲喂的CKD-3小鼠中通过CKD对循环激活素-A的诱导。附图31B描绘了在如本文描述的Alport's综合征小鼠中CKD对循环激活素-A的诱导。附图31C描绘了小鼠肾脏中抑制素betaA(Inhba)mRNA表达(激活素-A是抑制素betaA的同二聚体形成的)。附图31D描绘了用于肾脏组织匀浆中的抑制素β-A的westerns。附图31E描绘了附图31D的免疫印迹定量,免疫印迹定量的n=6;*p<0.05、**p<0.01、***p<0.005。

[0089] 附图32A显示了sham中的肾脏InhbA免疫染色。附图32B显示了CKD-3溶媒小鼠中的肾脏InhbA免疫染色。在Sham小鼠中，偶尔的管周间质细胞表达激活素-A，但是在CKD-3小鼠中，更多的管周间质细胞在不同强度下对于激活素-A是阳性的。比例尺50μm。

[0090] 附图33A和附图33B描绘了小鼠中类似于人3期肾病的慢性肾病。在小鼠模型中按照与人类CKD的分期类似的方式将CKD分期。如方法(章节9.10.1)中描述的带有消融性CKD的血管钙化模型用于产生与称为CKD-3的人类CKD 3期类似的降低的肾脏功能(溶媒治疗的为CKD-3 V)，通过菊糖清除和BUN来估计，与WT C57B6小鼠(WT)相比具有GFR的70％降低。10mg/kg每周两次皮下地用mActRIIA-Fc治疗CKD-3小鼠(CKD-3 R)对于菊糖清除或BUN没有影响。

[0091] 附图34描绘了来自Tsuchida等(Cell Comm.and Signaling,2009)的ActRIIA信号转导的示意图。激活素、肌肉生长抑制素和GDF11结合II型受体，ActRIIA(主要对于激活素)和ActRIIB(主要对于肌肉生长抑制素和GDF11)活化信号转导途径，包括通过smad 2,3的典型途径。

[0092] 附图35A和附图35B显示了CKD-3溶媒治疗的小鼠的肾脏(肾脏三色染色)。附图35C和附图35D显示了CKD-3 mActRIIA-Fc治疗的小鼠的肾脏(肾脏三色染色)。比例尺1mm。来自电灼损伤的皮层表面瘢痕由箭头标出。箭头指向了附图36中拍摄高功率显微照片的点。比例尺1mm。

[0093] 附图36A描绘了与WT和sham小鼠相比，在CKD-3小鼠中循环卵泡抑素水平没有改变。附图36B描绘了与WT和sham小鼠相比，在CKD-3小鼠中循环卵泡抑素样3(Fstl3)水平没有改变。

[0094] 附图37描绘了CKD-3小鼠的组织中激活素受体相互作用蛋白Arip1和Arip2)的水平。Arip1在CKD-3小鼠的残余肾脏中强烈表达，是Arip2表达的两倍。Arip1在CKD-3小鼠的主动脉中以低水平表达，而主动脉中的Arip2表达是Arip1的两倍。

[0095] 8.详细说明

[0096] 8.1 概述

[0097] 在一个方面，本文提供的是治疗和/或预防心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病、和/或与肾病相关和/或由肾病引起的心血管疾病、动脉硬化水平升高(例如，通过降低的血管顺应性所指示的)、和/或左心室肥大(LVH)，包括与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病和/或LVH的方法，其中所述方法包括向需要治疗和/或预防的对象施用ActRII信号转导抑制剂(例如，激活素配体陷阱)。在某些实施方式中，所述对象是肾脏的对象。所述ActRII信号转导抑制剂可以是ActRIIA信号转导和/或ActRIIB信号转导的抑制剂。

[0098] 特别地，本文提供的是治疗和/或预防心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病、与肾病相关和/或由肾病引起的心血管疾病、动脉硬化水平升高和/或左心室肥大(LVH)的方法，使用Snail、phosphosmad2、Dkk1、collal、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性作为患者群体的指示、作为对象对用ActRII信号转导抑制剂治疗和/或预防的响应性的指示、作为用ActRII信号转导抑制剂治疗的效力的指示或作为用ActRII信号转导抑制剂治疗的适当剂量的指

示。Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha也可以用于鉴定用ActRII信号转导抑制剂治疗和/或预防的疾病和/或状况。本文描述的方法中使用的ActRII信号转导抑制剂可以是ActRIIA信号转导和/或ActRIIB信号转导的抑制剂,例如,本文描述的或本领域已知的任何抑制剂。在优选的实施方式中,ActRII信号转导抑制剂是人源化的融合蛋白,其由ActRIIA的细胞外结构域和人类IgG1Fc结构域组成("ActRIIA-Fc,"例如,SEQ ID NO:7)。

[0099] 本文提供的方法部分地基于以下发现,与野生型小鼠相比,在慢性肾病诱导的血管钙化的小鼠模型中,Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和Osterix的水平提高,Klotho、alpha-SMA、MYOCD、ActRIIA和Sm22-alpha的水平降低。进一步的,不受理论的限制,mActRIIA-Fc的配体捕获降低了慢性肾病小鼠模型(ldlr-/-小鼠,高脂肪饮食)中的血管钙化。如本文呈现的实施例中所显示的(参见章节9),用mActRIIA-Fc治疗慢性肾病小鼠降低了血管钙化、降低了主动脉的钙水平、降低了phosphosmad3水平、降低了尿蛋白水平、降低了phosphosmad2水平、降低了Dkk1水平、降低了Runx2水平、降低了Alp水平、降低了BSAP水平、降低了CTX水平以及降低了Osterix水平,并提高了Klotho水平、提高了alpha-SMA水平、提高了Axin2水平和提高了Sm22-alpha水平。此外,本文提供的方法部分地基于以下发现,在用mActRIIA-Fc治疗时在慢性肾病小鼠中血管钙化的降低与Dkk1、phosphosmad2、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix水平的降低相关,以及与Klotho、alpha-SMA、Axin2和/或Sm22-alpha水平的提高相关(参见实施例,章节9)。合起来,本文呈现的数据表明,Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平可以鉴定哪些对象可能响应ActRIIA-Fc,和/或可以用于监测对ActRII信号转导抑制剂的临床响应,和/或选择目标患者群体。本文呈现的数据还表明,ActRIIA-Fc(例如,mActRIIA-Fc或ActRIIA-hFc,例如SEQ ID NO:7)在治疗与慢性肾病相关的血管钙化中是有用的。另外,本文呈现的数据还表明,ActRIIA-Fc(例如,mActRIIA-Fc或ActRIIA-hFc例如SEQ ID NO:7)在治疗与Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的水平升高相关的疾病,或与Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平降低相关的疾病方面是有用的。最后,本文呈现的数据还表明,ActRIIA-Fc(例如,mActRIIA-Fc或hActRIIA-hFc,例如SEQ ID NO:7)在降低主动脉粥样化中的钙沉积、降低主动脉钙水平、逆转CKD诱导的心脏重量增加、以及降低肾脏纤维化方面是有用的,因而,在治疗心血管疾病方面是有用的。

[0100] 实施例(参见章节9)中说明的、本文描述的发现表明,检测Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2或Sm22-alpha的水平和/或活性可以用作(i)对象的血管钙化程度的标志物(例如,血清标志物),(ii)测量对象对ActRII信号转导抑制剂(例如ActRIIA-Fc)的响应的标志物,或(iii)评估治疗之后对象种ActRII信号转导抑制剂(例如ActRIIA-Fc)的药效,其中所述对象是患有心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病、和/或与肾病相关和/或由肾病引起的心血管疾

病的对象。因而，在某些实施方式中，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、Axin2和/或Sm22-alpha可以用于本文描述的方法，作为ActRIIA-Fc(例如，ActRIIA-hFc，如SEQ ID NO:7)治疗的效力的指示物，和/或作为对ActRIIA-Fc(例如，ActRIIA-hFc，如SEQ ID NO:7)治疗没有响应的指示物。此外，如本文描述的，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha可以用作可靠的分子标志物来评估ActRIIA-Fc(例如，ActRIIA-hFc，如SEQ ID NO:7)的时间-过程治疗效力。进一步，在具体的实施方式中，本文提供的是一种方法，其包括检测血液中Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性(例如，检测与受损的血管平滑肌细胞功能相关的疾病中的异常表达)，以及以对Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的活性和/或水平剂量依赖性的方式施用ActRII信号转导抑制剂，例如，ActRIIA-Fc。

[0101] 在某些实施方式中，所述ActRII信号转导抑制剂是Sotatercept(SEQ ID NO:7；参见，例如，章节9)。Sotatercept是一种激活素配体陷阱，其在本文描述的方法中是有用的(参见章节8)。

[0102] 8.2 缩写

[0103] 如本文使用的，“Snai 1”或“Snail”是指snail同源物1。参见，例如Twigg and Wilkie,1999,Hum Genet.105(4):320-326,GenBank™登记号NM_005985.3提供了示范性的人类Snail核酸序列。GenBank™登记号NP_005976.2提供了示范性的人类Snail氨基酸序列。

[0104] 如本文使用的，“phosphosmad3”是指针对decapentaplegic同源物3的磷酸化的母本(phosphorylated mothers against decapentaplegic homolog 3)。参见，例如Matsuzaki,2013,Cytokine Growth Factor Rev,24(4):385-399。GenBank™登记号NM_005902.3提供了示范性的人类phosphosmad3核酸序列。GenBank™登记号NP_005893.1提供了示范性的人类phosphosmad3氨基酸序列。

[0105] 如本文使用的，“phosphosmad2”是指针对decapentaplegic同源物2的磷酸化的母本。参见，例如Matsuzaki,2013,Cytokine Growth Factor Rev,24(4):385-399。GenBank™登记号NM_001003652.3提供了示范性的人类phosphosmad2核酸序列。GenBank™登记号NP_001003652.1提供了示范性的人类phosphosmad2氨基酸序列。

[0106] 如本文使用的，“Dkk1”是指dickkopf相关蛋白1。参见，例如Fang,Y.,et al.,2014.J Am Soc Nephrol 25:1760-1763。GenBank™登记号NM_012242.2提供了示范性的人类Dkk1核酸序列。GenBank™登记号NP_036374.1提供了示范性的人类Dkk1氨基酸序列。

[0107] 如本文使用的，“colla1”是指1型胶原蛋白alpha 1。参见，例如Korkko et al.,1998,Am.J.Hum.Genet.,62:98-110。GenBank™登记号XM_005257059.2提供了示范性的人类colla1核酸序列。GenBank™登记号XP_005257116.2提供了示范性的人类colla1氨基酸序列。

[0108] 如本文使用的，“Runx2”是指runt相关的转录因子2。参见，例如Komori,2010,Adv

Exp Med Biol,658:43-49。GenBank™登记号NM_001145920.2提供了示范性的人类Runx2核酸序列。GenBank™登记号NP_001139392.1提供了示范性的人类Runx2氨基酸序列。

[0109] 如本文使用的，“Alp”或“Alpl”是指碱性磷酸酶。如本文使用的，“BSAP”是指骨骼特异性碱性磷酸酶。参见，例如Martins et al.,2013,Bone,56(2):390-397。GenBank™登记号NM_000478.4提供了示范性的人类Alp核酸序列。GenBank™登记号NP_000469.3提供了示范性的人类Alp氨基酸序列。

[0110] 如本文使用的，“CTX”是指I型胶原蛋白的C-末端端肽。参见，例如Rosen et al,2000,Calcif Tissue Int.66(2):100-103。

[0111] 如本文使用的，“Osterix”是指Osterix，也称为Sp7转录因子。参见，例如Cao et al.,2005,Cancer Res.65(4):1124-1128。GenBank™登记号NM_001173467.2提供了示范性的人类Osterix核酸序列。GenBank™登记号NP_001166938.1提供了示范性的人类Osterix氨基酸序列。

[0112] 如本文使用的，“Klotho”是指Klotho。参见，例如Matsumura et al.1998,Biochem Biophys Res Commun,242:626-630。GenBank™登记号NM_004795.3提供了示范性的人类Klotho核酸序列。GenBank™登记号NP_004786.2提供了示范性的人类Klotho氨基酸序列。

[0113] 如本文使用的，“alpha-SMA”或“αSMA”或“α-SMA”是指alpha平滑肌肌动蛋白。参见，例如Nowak et al.,1999,Nat.Genet.23:208-212。GenBank™登记号NM_001100.3提供了示范性的人类alpha-SMA核酸序列。GenBank™登记号NP_001091.1提供示范性的人类alpha-SMA氨基酸序列。

[0114] 如本文使用的，“MYOCD”是指心肌素。参见，例如Imamura et al.,2010,Gene,464:1-10。GenBank™登记号NM_001146312.2提供了示范性的人类MYOCD核酸序列。GenBank™登记号NP_001139784.1提供了示范性的人类MYOCD氨基酸序列。

[0115] 如本文使用的，“Sm22-alpha”或“Sm22α”或“Sm22-α”是指平滑肌蛋白22alpha，也称为“transgelin”、“Tagln”或“Tagln1”。参见，例如Camoretti-Mercado,1998,Genomics,49:452-457。GenBank™登记号NM_001001522.1提供了示范性的人类Sm22-alpha核酸序列。GenBank™登记号NP_001001522.1提供了示范性的人类Sm22-alpha氨基酸序列。

[0116] 如本文使用的，“ActRII”是指激活素II型受体。如本文使用的，“ActRIIA”是指激活素IIA型受体。参见，例如Mathews and Vale,1991,Cell 65:973-982。GenBank™登记号NM_001278579.1提供了示范性的人类ActRIIA核酸序列。GenBank™登记号NP_001265508.1提供了示范性的人类ActRIIA氨基酸序列。如本文使用的，“ActRIIB”是指激活素IIB型受体。参见，例如Attisano et al.,1992,Cell 68:97-108。GenBank™登记号NM_001106.3提供了示范性的人类ActRIIB核酸序列。GenBank™登记号NP_001097.2提供了示范性的人类ActRIIB氨基酸序列。

[0117] 如本文使用的，“Axin2”是指轴抑制剂2(axis inhibitor 2)。GenBank™登记号NM_004655.3提供了示范性的人类Axin2核酸序列。GenBank™登记号NP_004646.3提供了示范性的人类氨基酸序列。

[0118] 如本文使用的，“mActRIIA-Fc”或“ActRIIA-mFc”是指小鼠激活素IIA型受体-IgG1融合蛋白。参见，例如，美国专利No.8,173,601。如本文使用的，“mActRIIB-Fc”或“ActRIIB-mFc”是指小鼠激活素IIB型受体-IgG1融合蛋白。参见，例如，美国专利No.8,173,601。如本

文使用的，“"hActRIIA-Fc"或"ActRIIA-hFc"是指人类激活素IIA型受体-IgG1融合蛋白。参见，例如，美国专利No.8,173,601。如本文使用的，"hActRIIB-Fc"或"ActRIIB-hFc"是指人类激活素IIB型受体-IgG1融合蛋白。参见，例如，美国专利No.8,173,601。

[0119] 如本文使用的，"LVH"是指左心室肥大。

[0120] 8.3 治疗和/或预防的方法

[0121] 8.3.1 心血管疾病和/或血管钙化

[0122] 在某些实施方式中，本文提供的是治疗和/或预防对象的心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病、与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病、动脉硬化水平升高、左心室肥大、与左心室肥大相关和/或由左心室肥大引起的心血管疾病、和/或与肾病相关和/或由肾病引起的心血管疾病，包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂(例如，激活素配体陷阱)。在某些实施方式中，所述对象是肾脏的对象。在某些其他实施方式中，所述对象被诊断为患有慢性肾病-矿物质骨骼失调。在某些其他实施方式中，所述对象没有被诊断为患有慢性肾病-矿物质骨骼失调。

[0123] 在某些实施方式中，所述对象具有与参考群体中Runx2的水平和/或活性相比升高的Runx2水平和/或活性，与参考群体中Alp的水平和/或活性相比升高的Alp水平和/或活性，与参考群体中Snai1的水平和/或活性相比升高的Snai1水平和/或活性，与参考群体中phosphosmad2的水平和/或活性相比升高的phosphosmad2水平和/或活性，与参考群体中中phosphosmad3的水平和/或活性相比升高的phosphosmad3水平和/或活性，与参考群体中中尿蛋白的水平和/或活性相比升高的尿蛋白水平和/或活性，与参考群体中中Dkk1的水平和/或活性相比升高的Dkk1水平和/或活性，与参考群体中中激活素(例如，游离激活素)的水平和/或活性相比升高的激活素(例如，游离激活素)水平和/或活性，与参考群体中col1a1的水平和/或活性相比升高的col1a1水平和/或活性，与参考群体中BSAP的水平和/或活性相比升高的BSAP水平和/或活性，与参考群体中CTX的水平和/或活性相比升高的CTX水平和/或活性，与参考群体中Osterix的水平和/或活性相比升高的Osterix水平和/或活性，与参考群体中Klotho的水平和/或活性相比降低的Klotho水平和/或活性，与参考群体中alpha-SMA的水平和/或活性相比降低的alpha-SMA水平和/或活性，与参考群体中MYOCD的水平和/或活性相比降低的MYOCD水平和/或活性，与参考群体中Sm22-alpha的水平和/或活性相比降低的Sm22-alpha水平和/或活性；和/或与参考群体中ActRIIA的水平和/或活性相比降低的ActRIIA水平和/或活性。

[0124] 在某些实施方式中，所述对象具有与所述对象中在先的Runx2水平和/或活性相比升高的Runx2水平和/或活性，与所述对象中在先的Alp水平和/或活性相比升高的Alp水平和/或活性，与所述对象中在先的Snai1水平和/或活性相比升高的Snai1水平和/或活性，与所述对象中在先的phosphosmad2水平和/或活性相比升高的phosphosmad2水平和/或活性，与所述对象中在先的phosphosmad3水平和/或活性相比升高的phosphosmad3水平和/或活性，与所述对象中在先的尿蛋白水平和/或活性相比升高的尿蛋白水平和/或活性，与所述对象中在先的Dkk1水平和/或活性相比升高的Dkk1水平和/或活性，与所述对象中在先的激活素(例如，游离激活素)水平和/或活性相比升高的激活素(例如，游离激活素)水平和/或活性，与所述对象中在先的col1a1水平和/或活性相比升高的col1a1水平和/或活性，与所

述对象中在先的BSAP水平和/或活性相比升高的BSAP水平和/或活性，与所述对象中在先的CTX水平和/或活性相比升高的CTX水平和/或活性，与所述对象中在先的Osterix水平和/或活性相比升高的Osterix水平和/或活性，与所述对象中在先的Klotho水平和/或活性相比降低的Klotho水平和/或活性，与所述对象中在先的alpha-SMA水平和/或活性相比降低的alpha-SMA水平和/或活性，与所述对象中在先的MYOCD水平和/或活性相比降低的MYOCD水平和/或活性，与所述对象中在先的ActRIIA水平和/或活性相比降低的ActRIIA水平和/或活性，和/或与所述对象中在先的Sm22-alpha水平和/或活性相比降低的Sm22-alpha水平和/或活性。在某些实施方式中，所述对象中在先的Runx2水平和/或活性、所述对象中在先的Alp水平和/或活性、所述对象中在先的Snail水平和/或活性、所述对象中在先的phosphosmad2水平和/或活性、所述对象中在先的phosphosmad3水平和/或活性、所述对象中在先的尿蛋白水平和/或活性、所述对象中在先的Dkk1水平和/或活性、所述对象中在先的激活素(例如，游离激活素)水平和/或活性、所述对象中在先的colla1水平和/或活性、所述对象中在先的BSAP水平和/或活性、所述对象中在先的CTX水平和/或活性、所述对象中在先的Osterix水平和/或活性、所述对象中在先的Klotho水平和/或活性、所述对象中在先的alpha-SMA水平和/或活性、所述对象中在先的MYOCD水平和/或活性、所述对象中在先的ActRIIA水平和/或活性、所述对象中在先的Sm22-alpha水平和/或活性是(i)心血管疾病；(ii)血管钙化；(iii)与血管钙化相关和/或由血管钙化引起的心血管疾病；(iv)与肾病相关和/或由肾病引起的心血管疾病；(v)动脉硬化水平升高；和/或左心室肥大(LVH)的症状发作或诊断之前1、2、3、4、5、6、8、10、12、18、24或48个月的相应水平。

[0125] 在某些实施方式中，所述对象根据8.3.4中描述的方法治疗。在某些实施方式中，所述对象的血管钙化通过如章节8.6中描述的测定Agatston评分来分析。

[0126] 在某些实施方式中，用本文提供的方法治疗的对象具有如章节8.6中描述的Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性。因而，在某些具体的实施方式中，本文提供的方法包括(i)根据如章节8.6中描述的Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA和/或Sm22-alpha的水平和/或活性选择对象；和(ii)施用药学上有效剂量的ActRII信号转导抑制剂(例如，激活素配体陷阱)。在具体的实施方式中，所述对象患有心血管疾病。在具体的实施方式中，所述对象患有血管钙化。在具体的实施方式中，所述对象患有与血管钙化相关和/或由血管钙化引起的心血管疾病。在具体的实施方式中，所述对象患有与肾病相关和/或由肾病引起的心血管疾病。在具体的实施方式中，所述对象患有肾病。在某些其他实施方式中，所述对象被诊断为患有慢性肾病-矿物质骨骼失调。在某些其他实施方式中，所述对象没有被诊断为患有慢性肾病-矿物质骨骼失调。在某些实施方式中，所述对象的血管钙化通过如章节8.6中描述的测定Agatston评分来分析。在具体的实施方式中，所述对象被诊断患有动脉硬化水平升高。在具体的实施方式中，所述对象被诊断患有LVH。在具体的实施方式中，所述对象患有与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病。在具体的实施方式中，所述对象患有与LVH相关和/或由LVH引起的心血管疾病。

[0127] 在某些实施方式中，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活

素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性如章节8.6中描述的测定。在某些实施方式中,Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别是Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的蛋白质水平和/或活性。在某些实施方式中,Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别是Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的mRNA水平和/或活性。在某些实施方式中,Snail、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD和/或ActRIIA、Axin2、alpha-SMA水平是在组织中的。在某些实施方式中,所述组织是主动脉。在某些实施方式中,所述组织是肾脏。在某些实施方式中,所述组织是骨骼。在某些实施方式中,所述组织是血清。在优选的实施方式中,Runx2、Dkk1、Alp、osterix、sm22-alpha、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2水平和/或活性是在主动脉中的。在优选的实施方式中,phosphosmad2、phosphosmad3、尿蛋白、ActRIIA、Axin2和colla1的水平和/或活性是在肾脏中的。在优选的实施方式中,所述激活素水平和/或活性是在血清中的。在某些实施方式中,相对于参考群体,激活素的水平在所述对象中升高。在某些实施方式中,所述对象中卵泡抑素的水平约等于参考群体中卵泡抑素的水平。

[0128] 在某些实施方式中,所述参考群体是章节8.6中描述的群体。在某些实施方式中,所述对象是章节8.4中描述的对象。

[0129] 在某些实施方式中,所述ActRII信号转导抑制剂是ActRIIA-Fc,例如ActRIIA-hFc(例如,SEQ ID NO:7)。在某些实施方式中,所述ActRII信号转导抑制剂是如章节8.5中描述的ActRII信号转导抑制剂。

[0130] 在某些实施方式中,所述药学上有效的剂量是如章节8.7中描述的剂量。在某些实施方式中,所述药学上有效的剂量是初始剂量。在某些实施方式中,所述药学上有效的剂量以如章节8.7中描述的频率施用。在某些实施方式中,所述药学上有效的剂量如章节8.7中描述的施用。

[0131] 在某些实施方式中,如章节8.8中描述的,本文提供的方法与第二药学活性试剂组合使用。

[0132] 在某些实施方式中,在心血管疾病或慢性肾病的上下文中“治疗(treat)”、“治疗(treatment)”或“治疗(treating)”分别包括改善心血管疾病或慢性肾病的至少一种症状。

[0133] 本领域技术人员将认识到,Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2、Sm22-alpha、phosphosmad3和尿蛋白的水平和/或活性可以分别与相应参考群体中的水平和/或活性比较。因而,在某些实施方式中,Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2

和尿蛋白的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的两种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的三种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的三种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的四种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的五种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的六种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的七种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的八种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的九种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的十种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的十一种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的十二种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的十三种的水平和/或活性与相应参考群体中它们

的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snai1、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的十四种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snai1、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的十五种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snai1、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的十六种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snai1、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的十七种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。因而，在某些实施方式中，Runx2、Alp、Snai1、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白的每一种的水平和/或活性与相应参考群体中它们的水平和/或活性比较。

[0134] 8.3.2 与SNAI1、PHOSPHOSMAD2、DKK1、COL1A1、激活素(例如，游离激活素)、RUNX2、ALP、BSAP、CTX、OSTERIX、KLOTHO、ALPHA-SMA、MYOCD、PHOSPHOSMAD3、尿蛋白、ACTRIIA、AXIN2和/或SM22-ALPHA相关的疾病

[0135] 在某些实施方式中，本文提供的是治疗和/或预防与Snai1、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha相关的一种或更多种疾病的方法，包括向对象施用药学上有效剂量的ActRII信号转导抑制剂(例如，激活素配体陷阱)。

[0136] 在某些实施方式中，所述对象是章节8.4中描述的对象。

[0137] 在某些实施方式中，所述ActRII信号转导抑制剂是ActRIIA-Fc，例如ActRIIA-hFc(例如，SEQ ID NO:7)。在某些实施方式中，所述ActRII信号转导抑制剂是如章节8.5中描述的ActRII信号转导抑制剂。

[0138] 在某些实施方式中，所述药学上有效的剂量是如章节8.7中描述的剂量。在某些实施方式中，所述药学上有效的剂量是初始剂量。在某些实施方式中，所述药学上有效的剂量以如章节8.7中描述的频率施用。在某些实施方式中，所述药学上有效的剂量如章节8.7中描述的施用。在某些实施方式中，所述药学上有效的剂量取决于本文描述的某些生物标记物的水平和/或活性来调整。

[0139] 在某些实施方式中，如章节8.8中描述的，本文提供的方法与第二药学活性试剂组合使用。

[0140] 8.3.3 慢性肾病-矿物质/骨骼失调

[0141] 在某些实施方式中，本文提供的是治疗对象的慢性肾病-矿物质骨骼失调的方法，包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂(例如，激活素配体陷阱)。在某些实施方式中，提供的是降低对象中的骨吸收的方法，包括向所述对象施用药学上有

效剂量的ActRII信号转导抑制剂。在某些实施方式中，所述对象患有心血管疾病。在某些实施方式中，所述对象患有血管钙化。在某些实施方式中，所述对象患有与血管钙化相关和/或由血管钙化引起的心血管疾病。在某些实施方式中，所述对象患有与肾病相关和/或由肾病引起的心血管疾病。在某些实施方式中，所述对象是肾脏的对象。在某些其他实施方式中，所述对象被诊断为患有慢性肾病-矿物质骨骼失调。在某些其他实施方式中，所述对象没有被诊断为患有慢性肾病-矿物质骨骼失调。

[0142] 在某些实施方式中，所述对象具有与参考群体中Runx2的水平和/或活性相比升高的Runx2水平和/或活性，与参考群体中Alp的水平和/或活性相比升高的Alp水平和/或活性，与参考群体中Snail的水平和/或活性相比升高的Snail水平和/或活性，与参考群体中phosphosmad2的水平和/或活性相比升高的phosphosmad2水平和/或活性，与参考群体中phosphosmad3的水平和/或活性相比升高的phosphosmad3水平和/或活性，与参考群体中尿蛋白的水平和/或活性相比升高的尿蛋白水平和/或活性，与参考群体中Dkk1的水平和/或活性相比升高的Dkk1水平和/或活性，与参考群体中col1a1的水平和/或活性相比升高的col1a1水平和/或活性，与参考群体中激活素(例如，游离激活素)的水平和/或活性相比升高的激活素(例如，游离激活素)水平和/或活性，与参考群体中BSAP的水平和/或活性相比升高的BSAP水平和/或活性，与参考群体中CTX的水平和/或活性相比升高的CTX水平和/或活性，与参考群体中Osterix的水平和/或活性相比升高的Osterix水平和/或活性，与参考群体中Klotho的水平和/或活性相比降低的Klotho水平和/或活性，与参考群体中alpha-SMA的水平和/或活性相比降低的alpha-SMA水平和/或活性，与参考群体中MYOCD的水平和/或活性相比降低的MYOCD水平和/或活性，与参考群体中ActRIIA的水平和/或活性相比降低的ActRIIA水平和/或活性，和/或与参考群体中Sm22-alpha的水平和/或活性相比降低的Sm22-alpha水平和/或活性。在某些实施方式中，Snail、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2、ActRIIA、Axin2和/或alpha-SMA水平是组织中的。在某些实施方式中，所述组织是主动脉。在某些实施方式中，所述组织是肾脏。在某些实施方式中，所述组织是骨骼。在某些实施方式中，所述组织是血清。在优选的实施方式中，Runx2、Dkk1、Alp、osterix、sm22-alpha、Klotho、alpha-SMA、MYOCD和/或ActRIIA水平和/或活性是在主动脉中的。在优选的实施方式中，phosphosmad2、phosphosmad3、尿蛋白和col1a1和/或ActRIIA水平和/或活性是在肾脏中的。在优选的实施方式中，所述激活素水平和/或活性是在血清中的。在某些实施方式中，Snail、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、ActRIIA和/或alpha-SMA水平是在主动脉中的。

[0143] 在某些实施方式中，所述对象具有与对象中在先的Runx2水平和/或活性相比升高的Runx2水平和/或活性，与对象中在先的Alp水平和/或活性相比升高的Alp水平和/或活性，与对象中在先的Snail水平和/或活性相比升高的Snail水平和/或活性，与对象中在先的phosphosmad2水平和/或活性相比升高的phosphosmad2水平和/或活性，与对象中在先的phosphosmad3水平和/或活性相比升高的phosphosmad3水平和/或活性，与对象中在先的尿蛋白水平和/或活性相比升高的尿蛋白水平和/或活性，与对象中在先的Dkk1水平和/或活性相比升高的Dkk1水平和/或活性，与对象中在先的激活素(例如，游离激活素)水平和/或

活性相比升高的激活素(例如,游离激活素)水平和/或活性,与所述对象中在先的collal水平和/或活性相比升高的collal水平和/或活性,与所述对象中在先的BSAP水平和/或活性相比升高的BSAP水平和/或活性,与所述对象中在先的CTX水平和/或活性相比升高的CTX水平和/或活性,与所述对象中在先的Osterix水平和/或活性相比升高的Osterix水平和/或活性,与所述对象中在先的Klotho水平和/或活性相比降低的Klotho水平和/或活性,与所述对象中在先的alpha-SMA水平和/或活性相比降低的alpha-SMA水平和/或活性,与所述对象中在先的MYOCD水平和/或活性相比降低的MYOCD水平和/或活性,与所述对象中在先的ActRIIA水平和/或活性相比降低的ActRIIA水平和/或活性,和/或与所述对象中在先的Sm22-alpha水平和/或活性相比降低的Sm22-alpha水平和/或活性。在某些实施方式中,所述对象中在先的Runx2水平和/或活性、所述对象中在先的Alp水平和/或活性、所述对象中在先的Snail水平和/或活性、所述对象中在先的phosphosmad2水平和/或活性、所述对象中在先的phosphosmad3水平和/或活性、所述对象中在先的尿蛋白水平和/或活性、所述对象中在先的Dkk1水平和/或活性、所述对象中在先的激活素(例如,游离激活素)水平和/或活性、所述对象中在先的collal水平和/或活性、所述对象中在先的BSAP水平和/或活性、所述对象中在先的CTX水平和/或活性、所述对象中在先的Osterix水平和/或活性、所述对象中在先的Klotho水平和/或活性、所述对象中在先的alpha-SMA水平和/或活性、所述对象中在先的MYOCD水平和/或活性、所述对象中在先的ActRIIA水平和/或活性、所述对象中在先的Sm22-alpha水平和/或活性是CKD-MBD的症状发作或诊断之前1、2、3、4、5、6、8、10、12、18、24或48个月的相应水平。

[0144] 在某些实施方式中,所述对象根据章节8.3.4中描述的方法治疗。在某些实施方式中,所述对象的血管钙化通过如章节8.6中描述的测定Agatston评分来分析。

[0145] 在某些实施方式中,用本文提供的方法治疗的对象具有如章节8.6中描述的Snail、phosphosmad2、Dkk1、collal、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性。因而,在某些具体的实施方式中,本文提供的方法包括(i)根据如章节8.6中描述的Snail、phosphosmad2、Dkk1、collal、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA和/或Sm22-alpha的水平和/或活性选择对象;和(ii)施用药学上有效剂量的ActRII信号转导抑制剂(例如,激活素配体陷阱)。在具体的实施方式中,所述对象患有心血管疾病。在具体的实施方式中,所述对象患有血管钙化。在具体的实施方式中,所述对象患有与血管钙化相关和/或由血管钙化引起的心血管疾病。在具体的实施方式中,所述对象患有与肾病相关和/或由肾病引起的心血管疾病。在具体的实施方式中,所述对象患有肾病。在某些其他实施方式中,所述对象被诊断为患有慢性肾病-矿物质骨骼失调。在某些其他实施方式中,所述对象没有被诊断为患有慢性肾病-矿物质骨骼失调。在具体的实施方式中,所述对象患有动脉硬化水平升高。在具体的实施方式中,所述对象患有与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病。在具体的实施方式中,所述对象患有LVH。在具体的实施方式中,所述对象患有与LVH相关和/或由LVH引起的心血管疾病。

[0146] 在某些实施方式中,Snail、phosphosmad2、Dkk1、collal、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、

ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性如章节8.6中描述的测定。在某些实施方式中，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的升高的水平是如章节8.6中描述的。在某些实施方式中，Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的降低的水平是如章节8.6中描述的。在某些实施方式中，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2、ActRIIA、Axin2和/或alpha-SMA水平是组织中的。在某些实施方式中，所述组织是主动脉。在某些实施方式中，所述组织是肾脏。在某些实施方式中，所述组织是骨骼。在某些实施方式中，所述组织是血清。在优选的实施方式中，Runx2、Dkk1、Alp、osterix、sm22-alpha、Klotho、alpha-SMA、ActRIIA、Axin2和MYOCD水平和/或活性是在主动脉中的。在优选的实施方式中，phosphosmad2、phosphosmad3、尿蛋白、ActRIIA和colla1水平和/或活性是在肾脏中的。在优选的实施方式中，所述激活素水平和/或活性是在血清中的。在某些实施方式中，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA和/或Sm22-alpha的水平和/或活性分别是Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA和/或Sm22-alpha的蛋白质水平和/或活性。在某些实施方式中，Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA和/或Sm22-alpha的水平和/或活性分别是Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA和/或Sm22-alpha的mRNA水平和/或活性。在某些实施方式中，相对于参考群体，激活素的水平在所述对象中升高。在某些实施方式中，所述对象中卵泡抑素的水平约等于参考群体中卵泡抑素的水平。

[0147] 在某些实施方式中，所述参考群体是章节8.6中描述的群体。在某些实施方式中，所述对象是章节8.4中描述的对象。

[0148] 在某些实施方式中，所述ActRII信号转导抑制剂是ActRIIA-Fc，例如ActRIIA-hFc(例如，SEQ ID NO:7)。在某些实施方式中，所述ActRII信号转导抑制剂是如章节8.5中描述的ActRII信号转导抑制剂。

[0149] 在某些实施方式中，所述药学上有效的剂量是如章节8.7中描述的剂量。在某些实施方式中，所述药学上有效的剂量是初始剂量。在某些实施方式中，所述药学上有效的剂量以如章节8.7中描述的频率施用。在某些实施方式中，所述药学上有效的剂量如章节8.7中描述的施用。

[0150] 在某些实施方式中，如章节8.8中描述的，本文提供的方法与第二药学活性试剂组合使用。

[0151] 在某些实施方式中，本文提供的是治疗对象的末期肾病的方法，包括向所述对象施用药学上有效剂量的ActRII信号转导抑制剂(例如，激活素配体陷阱)，其中所述对象在血液透析，以及其中所述对象早先施用了促红细胞生成素刺激剂。在某些实施方式中，本文提供的是治疗对象的末期肾病的方法，包括以每14天一次的间隔时间向所述对象皮下地施

用0.13mg/kg的ActRII信号转导抑制剂(例如,激活素配体陷阱),其中所述对象在血液透析,以及其中所述对象早先施用了促红细胞生成素刺激剂。在某些实施方式中,本文提供的是治疗对象的末期肾病的方法,包括以每14天一次的间隔时间向所述对象皮下地施用0.26mg/kg的ActRII信号转导抑制剂(例如,激活素配体陷阱),其中所述对象在血液透析,以及其中所述对象早先施用了促红细胞生成素刺激剂。在某些实施方式中,本文提供的是治疗对象的末期肾病的方法,包括以每28天一次的间隔时间向所述对象皮下地施用0.3mg/kg的ActRII信号转导抑制剂(例如,激活素配体陷阱),其中所述对象在血液透析,以及其中所述对象早先施用了促红细胞生成素刺激剂。在某些实施方式中,本文提供的是治疗对象的末期肾病的方法,包括以每28天一次的间隔时间向所述对象皮下地施用0.5mg/kg的ActRII信号转导抑制剂(例如,激活素配体陷阱),其中所述对象在血液透析,以及其中所述对象早先施用了促红细胞生成素刺激剂。在某些实施方式中,本文提供的是治疗对象的末期肾病的方法,包括以每28天一次的间隔时间向所述对象皮下地施用0.7mg/kg的ActRII信号转导抑制剂(例如,激活素配体陷阱),其中所述对象在血液透析,以及其中所述对象早先施用了促红细胞生成素刺激剂。在某些实施方式中,本文提供的是治疗对象的末期肾病的方法,包括以每14天一次的间隔时间向所述对象静脉内地施用0.1mg/kg的ActRII信号转导抑制剂(例如,激活素配体陷阱),其中所述对象在血液透析,以及其中所述对象早先施用了促红细胞生成素刺激剂。在某些实施方式中,本文提供的是治疗对象的末期肾病的方法,包括以每14天一次的间隔时间向所述对象皮下地施用0.2mg/kg的ActRII信号转导抑制剂(例如,激活素配体陷阱),其中所述对象在血液透析,以及其中所述对象早先施用了促红细胞生成素刺激剂。在某些实施方式中,所述ActRII信号转导抑制剂是ActRIIA-hFc(SEQ ID NO:7)。

[0152] 8.3.4 调整的给药

[0153] Snai1、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性可以进一步用于(i)评估对象的适当的给药,其中所述对象是用ActRII信号转导抑制剂(例如,激活素配体陷阱)治疗的或将要治疗的候选者;(ii)评估在治疗期间是否调整ActRII信号转导抑制剂的剂量;和/或(iii)评估ActRII信号转导抑制剂的适当的维持剂量。如果Snai1、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha水平和/或活性大于或小于参考群体中的水平和/或活性,分别取决于Snai1、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性,用ActRII信号转导抑制剂的给药可以启动、提高、降低、延迟或终止。在某些实施方式中,本文提供的是治疗和/或预防对象的心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病、与肾病相关和/或由肾病引起的心血管疾病、CKD-MBD、动脉硬化水平升高、LVH、与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病和/或与LVH相关和/或由LVH引起的心血管疾病的方法,包括(i)向所述对象施用初始剂量的ActRII信号转导抑制剂;(ii)进行所述对象中Snai1、phosphosmad2、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、

Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性的第一次测量；(iii)一段时间以后，进行所述对象中Snai1、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性的第二次测量；和(v)向所述对象施用调整剂量的ActRII信号转导抑制剂。在某些实施方式中，所述ActRII信号转导抑制剂是ActRIIA-Fc，例如ActRIIA-hFc(例如，SEQ ID NO:7)。在某些实施方式中，所述ActRII信号转导抑制剂是如章节8.5中描述的ActRII信号转导抑制剂。在某些其他实施方式中，所述对象被诊断为患有慢性肾病-矿物质骨骼失调。在某些其他实施方式中，所述对象没有被诊断为患有慢性肾病-矿物质骨骼失调。在某些实施方式中，所述对象的血管钙化通过如章节8.6.6中描述的测定Agatston评分来分析。在某些实施方式中，所述调整的剂量是基于所述第一次测量和所述第二次测量之间检测到的变化。

[0154] 在某些实施方式中，Snai1、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性如章节8.6中描述的测定。在某些实施方式中，Snai1、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别是Snai1、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白和/或Sm22-alpha的蛋白质水平和/或活性。在某些实施方式中，Snai1、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别是Snai1、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或Sm22-alpha的mRNA水平和/或活性。在某些实施方式中，Snai1、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或alpha-SMA水平是组织中的。在某些实施方式中，所述组织是主动脉。在某些实施方式中，所述组织是肾脏。在某些实施方式中，所述组织是骨骼。在某些实施方式中，所述组织是血清。在优选的实施方式中，Runx2、Dkk1、Alp、osterix、sm22-alpha、Klotho、alpha-SMA、MYOCD、ActRIIA或Axin2和平和/或活性是在主动脉中的。在优选的实施方式中，phosphosmad2、phosphosmad3、尿蛋白、ActRIIA、Axin2和col1a1的水平和/或活性是在肾脏中的。在优选的实施方式中，所述激活素水平和/或活性是在血清中的。在某些实施方式中，相对于参考群体，激活素的水平在所述对象中升高。在某些实施方式中，相对于参考群体，卵泡抑素的水平在所述对象中是正常的。在某些实施方式中，所述对象的血管钙化通过如章节8.6中描述的测定Agatston评分来分析。

[0155] 在某些实施方式中，所述参考群体是章节8.6中描述的群体。在某些实施方式中，所述对象是章节8.4中描述的对象。

[0156] 在某些实施方式中，所述初始剂量是如章节8.7中描述的剂量。在某些实施方式中，所述初始剂量以如章节8.7中描述的频率施用。在某些实施方式中，所述初始剂量如章节8.7中描述的施用。

[0157] 在某些实施方式中，所述第一次测量和/或所述第二次测量如章节8.6中描述的进行。在某些实施方式中，所述第一次测量在治疗的开始之前进行。在某些实施方式中，所述第一次测量在治疗的开始之后，或在其最多1天、2天、3天、4天、5天、6天或1周、2周、3周、4周或两个月之内进行。在某些实施方式中，所述第二次测量在治疗开始后立即进行，或在其最多1天、2天、3天、4天、5天、6天或1周、2周、1个月、2个月、3个月、4个月、5个月、6个月、7个月、8个月、9个月、10个月、11个月或12个月之内进行。

[0158] 在某些实施方式中，如果与参考群体中Runx2的水平和/或活性相比Runx2的水平和/或活性升高，与参考群体中Alp的水平和/或活性相比Alp的水平和/或活性升高，与参考群体中Snai1的水平和/或活性相比Snai1的水平和/或活性升高，与参考群体中phosphosmad2的水平和/或活性相比phosphosmad2的水平和/或活性升高，与参考群体中phosphosmad3的水平和/或活性相比phosphosmad3的水平和/或活性升高，与参考群体中尿蛋白的水平和/或活性相比尿蛋白的水平和/或活性升高，与参考群体中Dkk1的水平和/或活性相比Dkk1的水平和/或活性升高，与参考群体中col1a1的水平和/或活性相比col1a1的水平和/或活性升高，与参考群体中激活素(例如，游离激活素)的水平和/或活性相比激活素(例如，游离激活素)的水平和/或活性升高，与参考群体中BSAP的水平和/或活性相比BSAP的水平和/或活性升高，与参考群体中CTX的水平和/或活性相比CTX的水平和/或活性升高，与参考群体中Osterix的水平和/或活性相比Osterix的水平和/或活性升高，与参考群体中Klotho的水平和/或活性相比Klotho的水平和/或活性降低，与参考群体中alpha-SMA的水平和/或活性相比alpha-SMA的水平和/或活性降低，与参考群体中MYOCD的水平和/或活性相比MYOCD的水平和/或活性降低，与参考群体中ActRIIA的水平和/或活性相比ActRIIA的水平和/或活性降低，与参考群体中Axin2的水平和/或活性相比Axin2的水平和/或活性降低，和/或与参考群体中Sm22-alpha的水平和/或活性相比Sm22-alpha的水平和/或活性降低，所述ActRII信号转导抑制剂的调整的剂量大于所述初始剂量。在某些实施方式中，Snail、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的升高的水平是如章节8.6中描述的。在某些实施方式中，Klotho、alpha-SMA、ALP、ActRIIA、Axin2和/或Sm22-alpha的降低的水平是如章节8.6中描述的。在某些实施方式中，Snail、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或alpha-SMA水平是组织中的。在某些实施方式中，所述组织是主动脉。在某些实施方式中，所述组织是肾脏。在某些实施方式中，所述组织是骨骼。在某些实施方式中，所述组织是血清。在优选的实施方式中，Runx2、Dkk1、Alp、osterix、sm22-alpha、Klotho、alpha-SMA、ActRIIA、Axin2和MYOCD水平和/或活性是在主动脉中的。在优选的实施方式中，phosphosmad2、phosphosmad3、尿蛋白、ActRIIA、Axin2和col1a1的水平和/或活性是在肾脏中的。在优选的实施方式中，所述激活素水平和/或活性是在血清中的。

[0159] 在某些实施方式中，所述调整的剂量是如章节8.7中描述的剂量。在某些实施方式中，所述调整的剂量以如章节8.7中描述的频率施用。在某些实施方式中，所述调整的剂量如章节8.7中描述的施用。

[0160] 在某些实施方式中，所述对象具有与对象中在先的Runx2水平和/或活性相比升高的Runx2水平和/或活性，与对象中在先的Alp水平和/或活性相比升高的Alp水平和/或活

性，与对象中在先的Snail水平和/或活性相比升高的Snail水平和/或活性，与对象中在先的phosphosmad2水平和/或活性相比升高的phosphosmad2水平和/或活性，与对象中在先的phosphosmad3水平和/或活性相比升高的phosphosmad3水平和/或活性，与对象中在先的尿蛋白水平和/或活性相比升高的尿蛋白水平和/或活性，与对象中在先的Dkk1水平和/或活性相比升高的Dkk1水平和/或活性，与对象中在先的激活素(例如，游离激活素)水平和/或活性相比升高的激活素(例如，游离激活素)水平和/或活性，与所述对象中在先的coll1a1水平和/或活性相比升高的coll1a1水平和/或活性，与所述对象中在先的BSAP水平和/或活性相比升高的BSAP水平和/或活性，与所述对象中在先的CTX水平和/或活性相比升高的CTX水平和/或活性，与所述对象中在先的Osterix水平和/或活性相比升高的Osterix水平和/或活性，与所述对象中在先的Klotho水平和/或活性相比降低的Klotho水平和/或活性，与所述对象中在先的alpha-SMA水平和/或活性相比降低的alpha-SMA水平和/或活性，与所述对象中在先的MYOCD水平和/或活性相比降低的MYOCD水平和/或活性，与所述对象中在先的ActRIIA水平和/或活性相比降低的ActRIIA水平和/或活性，与所述对象中在先的Axin2水平和/或活性相比降低的Axin2水平和/或活性，和/或与所述对象中在先的Sm22-alpha水平和/或活性相比降低的Sm22-alpha水平和/或活性。在某些实施方式中，所述对象中在先的Runx2水平和/或活性、所述对象中在先的Alp水平和/或活性、所述对象中在先的Snail水平和/或活性、所述对象中在先的phosphosmad2水平和/或活性、所述对象中在先的phosphosmad3水平和/或活性、所述对象中在先的尿蛋白水平和/或活性、所述对象中在先的Dkk1水平和/或活性、所述对象中在先的激活素(例如，游离激活素)水平和/或活性、所述对象中在先的coll1a1水平和/或活性、所述对象中在先的BSAP水平和/或活性、所述对象中在先的CTX水平和/或活性、所述对象中在先的Osterix水平和/或活性、所述对象中在先的Klotho水平和/或活性、所述对象中在先的alpha-SMA水平和/或活性、所述对象中在先的MYOCD水平和/或活性、所述对象中在先的ActRIIA水平和/或活性、所述对象中在先的ActRIIA水平和/或活性、所述对象中在先的Axin2水平和/或活性、所述对象中在先的Axin2水平和/或活性、和/或所述对象中在先的Sm22-alpha水平和/或活性是(i)心血管疾病；(ii)血管钙化；(iii)与血管钙化相关和/或由血管钙化引起的心血管疾病；(iv)与肾病相关和/或由肾病引起的心血管疾病；(v)CKD-MBD；(vi)动脉硬化水平升高；(vii)LVH；(viii)与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病；和/或(ix)与LVH相关和/或由LVH引起的心血管疾病的症状发作或诊断之前1、2、3、4、5、6、8、10、12、18、24或48个月的相应水平。

[0161] 在某些实施方式中，如果与参考群体中Runx2的水平和/或活性相比Runx2的水平和/或活性降低，与参考群体中Alp的水平和/或活性相比Alp的水平和/或活性降低，与参考群体中Snail的水平和/或活性相比Snail的水平和/或活性降低，与参考群体中phosphosmad2的水平和/或活性相比phosphosmad2的水平和/或活性降低，与参考群体中phosphosmad3的水平和/或活性相比phosphosmad3的水平和/或活性降低，与参考群体中尿蛋白的水平和/或活性相比尿蛋白的水平和/或活性降低，与参考群体中Dkk1的水平和/或活性相比Dkk1的水平和/或活性降低，与参考群体中coll1a1的水平和/或活性相比coll1a1的水平和/或活性降低，与参考群体中激活素(例如，游离激活素)的水平和/或活性相比激活素(例如，游离激活素)的水平和/或活性降低，与参考群体中BSAP的水平和/或活性相比

BSAP的水平和/或活性降低，与参考群体中CTX的水平和/或活性相比CTX的水平和/或活性降低，与参考群体中Osterix的水平和/或活性相比Osterix的水平和/或活性降低，与参考群体中Klotho的水平和/或活性相比Klotho的水平和/或活性升高，与参考群体中alpha-SMA的水平和/或活性相比alpha-SMA的水平和/或活性升高，与参考群体中MYOCD的水平和/或活性相比MYOCD的水平和/或活性升高，与参考群体中ActRIIA的水平和/或活性相比ActRIIA的水平和/或活性升高，与参考群体中Axin2的水平和/或活性相比Axin2的水平和/或活性升高，和/或与参考群体中Sm22-alpha的水平和/或活性相比Sm22-alpha的水平和/或活性升高，所述ActRII信号转导抑制剂(例如，激活素配体陷阱)的调整的剂量小于所述初始剂量。在某些实施方式中，Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的升高的水平是如章节8.6中描述的。在某些实施方式中，Snail、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的降低的水平是如章节8.6中描述的。在某些实施方式中，Snail、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、phosphosmad3、尿蛋白、ActRIIA、Axin2和/或alpha-SMA水平是组织中的。在某些实施方式中，所述组织是主动脉。在某些实施方式中，所述组织是肾脏。在某些实施方式中，所述组织是骨骼。在某些实施方式中，所述组织是血清。在优选的实施方式中，Runx2、Dkk1、Alp、osterix、sm22-alpha、Klotho、alpha-SMA、ActRIIA、Axin2和MYOCD水平和/或活性是在主动脉中的。在优选的实施方式中，phosphosmad2、phosphosmad3、尿蛋白、ActRIIA、Axin2和col1a1的水平和/或活性是在肾脏中的。在优选的实施方式中，所述激活素水平和/或活性是在血清中的。在某些实施方式中，Snail、phosphosmad2、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、Sm22-alpha、MYOCD、ActRIIA、Axin2和/或alpha-SMA水平是主动脉中的。

[0162] 在某些实施方式中，所述参考群体是章节8.6中描述的群体。在某些实施方式中，所述调整的剂量是如章节8.7中描述的剂量。在某些实施方式中，所述调整的剂量以如章节8.7中描述的频率施用。在某些实施方式中，所述调整的剂量如章节8.7中描述的施用。

[0163] 在某些实施方式中，如章节8.8中描述的，本文提供的方法与第二药学活性试剂组合使用。

[0164] 本领域技术人员将认识到，Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2、Sm22-alpha、phosphosmad3和尿蛋白的水平和/或活性可以分别与相应参考群体中的水平和/或活性独立比较，例如，如上文在章节8.3.1中描述的。

[0165] 8.4 患者群体

[0166] 根据本文描述的方法治疗的对象可以是任何哺乳动物，例如啮齿动物和灵长类，在优选的实施方式中，是人类。在某些实施方式中，本文描述的方法可以用于在任何哺乳动物例如啮齿动物和灵长类中，在优选的实施方式中，在人类对象中治疗心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病、与肾病相关和/或由肾病引起的心血管疾病、高周转ROD、动脉硬化水平升高、与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病、LVH和/或与LVH相关和/或由LVH引起的心血管疾病；或者监视和/或降低血管钙化、动脉的(例如，血管的)硬化、LVH、主动脉钙水平、内皮向间充质转变

和/或骨形成，和/或提高骨骼矿物密度和/或血管平滑肌细胞功能。

[0167] 在某些实施方式中，与参考群体中的激活素水平相比，根据本文描述的方法治疗的对象具有升高的激活素水平。在某些实施方式中，所述激活素水平是肾脏、血浆或主动脉的激活素水平。在某些实施方式中，所述激活素是激活素A。在某些实施方式中，与参考群体中的卵泡抑素水平相比，根据本文描述的方法治疗的对象具有大约相等的卵泡抑素水平。在某些实施方式中，与参考群体中的卵泡抑素水平相比，根据本文描述的方法治疗的对象不具有升高的卵泡抑素水平。在某些实施方式中，与参考群体中的卵泡抑素水平相比，根据本文描述的方法治疗的对象不具有降低的卵泡抑素水平。在某些实施方式中，与参考群体中的卵泡抑素样3水平相比，根据本文描述的方法治疗的对象具有大约相等的卵泡抑素样3水平。在某些实施方式中，与参考群体中的卵泡抑素样3水平相比，根据本文描述的方法治疗的对象不具有升高的卵泡抑素样3水平。在某些实施方式中，与参考群体中的卵泡抑素样3水平相比，根据本文描述的方法治疗的对象不具有降低的卵泡抑素样3水平。在某些实施方式中，与参考群体中的抑制素水平相比，根据本文描述的方法治疗的对象具有大约相等的抑制素水平。在某些实施方式中，与参考群体中的抑制素水平相比，根据本文描述的方法治疗的对象不具有升高的抑制素水平。在某些实施方式中，与参考群体中的抑制素水平相比，根据本文描述的方法治疗的对象不具有降低的抑制素水平。在某些实施方式中，对象中的游离激活素水平通过抑制素、卵泡抑素、卵泡抑素样3水平对激活素水平的化学当量来确定。因而，在某些实施方式中，根据本文描述的方法治疗的对象具有提高的游离激活素水平。

[0168] 在某些实施方式中，与章节8.6中描述的参考群体相比，根据本文描述的方法治疗的对象不具有phosphoerk 1/2的提高。在某些实施方式中，phosphoerk 1/2是主动脉的phosphoerk 1/2。

[0169] 在某些实施方式中，与参考群体中的EnMT相比，根据本文描述的方法治疗的对象具有提高的内皮向间充质转变(EnMT)。在某些实施方式中，根据本文描述的方法治疗的对象具有与EnMT相关的转录因子，例如Snail的提高的表达。在某些实施方式中，与参考群体中的成骨细胞转变相比，对象中提高的EnMT引起对象中提高的成骨细胞转变。在某些实施方式中，与参考群体中的血管硬化相比，对象中提高的EnMT引起对象中提高的血管硬化。在某些实施方式中，与参考群体中的血管钙化相比，对象中提高的EnMT引起对象中提高的血管钙化。在某些实施方式中，与参考群体中的血管平滑肌功能相比，对象中提高的EnMT引起对象中降低的血管平滑肌功能。在某些实施方式中，与参考群体中的LVH相比，对象中提高的EnMT引起对象中提高的LVH。

[0170] 在某些实施方式中，根据本文提供的方法治疗的对象患有肾脏纤维化。在某些实施方式中，根据本文提供的方法治疗的对象患有肾小球硬化症。

[0171] 在某些实施方式中，分别与参考群体中Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平相比，根据本文描述的方法治疗的对象具有升高的Snail、phosphosmad2、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、phosphosmad3、尿蛋白和/或Osterix的水平和/或活性，和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中，根据本文

描述的方法治疗的对象具有(i)血管钙化;和(ii)分别与参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平相比,升高的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性,和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中,根据本文描述的方法治疗的对象具有(i)心血管疾病;和(ii)分别与参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平相比,升高的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性,和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中,根据本文描述的方法治疗的对象具有(i)与血管钙化相关和/或由血管钙化引起的心血管疾病;和(ii)分别与参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平相比,升高的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性,和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中,根据本文描述的方法治疗的对象具有(i)与肾病相关和/或由肾病引起的心血管疾病;和(ii)分别与参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平相比,升高的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性,和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中,根据本文描述的方法治疗的对象具有(i)CKD-MBD;和(ii)分别与参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平相比,升高的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性,和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中,根据本文描述的方法治疗的对象具有(i)动脉硬化水平升高;和(ii)分别与参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平相比,升高的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性,和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中,根据本文描述的方法治疗的对象具有(i)与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病;和(ii)分别与参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、

ActRIIA和/或Sm22-alpha的水平相比，升高的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性，和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中，根据本文描述的方法治疗的对象具有(i)LVH；和(ii)分别与参考群体中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平相比，升高的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性，和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。在某些实施方式中，根据本文描述的方法治疗的对象具有(i)与LVH相关和/或由LVH引起的心血管疾病；和(ii)与参考群体中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的水平相比，升高的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性，和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。

[0172] 在某些实施方式中，根据本文描述的方法治疗的对象分别与在(i)心血管疾病；(ii)血管钙化；(iii)与血管钙化相关和/或由血管钙化引起的心血管疾病；(iv)与肾病相关和/或由肾病引起的心血管疾病；(v)CKD-MBD；(vi)动脉硬化水平升高；(vii)LVH；(viii)与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病；和/或(ix)与LVH相关和/或由LVH引起的心血管疾病的症状发作或诊断之前1、2、3、4、5、6、8、10、12、18、24或48个月之时，所述对象中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的在先水平相比，具有升高的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix水平和/或活性，和/或降低的Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha水平和/或活性。

[0173] 在某些其他实施方式中，所述对象被诊断为患有慢性肾病-矿物质骨骼失调。在某些其他实施方式中，所述对象没有被诊断为患有慢性肾病-矿物质骨骼失调。在某些实施方式中，所述参考群体是章节8.6中描述的群体。在某些实施方式中，Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的升高的水平是如章节8.6中描述的。在某些实施方式中，Klotho、alpha-SMA、MYOCD、ActRIIA和/或Sm22-alpha的降低的水平是如章节8.6中描述的。

[0174] 在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的Snail水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的phosphosmad2水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的phosphosmad3水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的尿蛋白水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的Dkk1水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的Colla1水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的Runx2水平相关的疾病。在某些实施方式中，根据本文提供的方法治

疗的对象患有与升高的Alp水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的BSAP水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的CTX水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的Osterix水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与升高的ActRIIA水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与降低的Klotho水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与降低的alpha-SMA水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与降低的MYOCD水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与降低的Sm22-alpha水平相关的疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有与降低的ActRIIA水平相关的疾病。

[0175] 在某些实施方式中，根据本文提供的方法治疗的对象患有心血管疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有血管钙化。在某些实施方式中，根据本文提供的方法治疗的对象患有与血管钙化相关和/或由血管钙化引起的心血管疾病。在某些实施方式中，所述血管钙化是如章节8.6中描述的确定的。在某些实施方式中，所述血管钙化是通过Agatston评分确定的。在某些实施方案中，所述血管钙化是钙化性动脉粥样硬化、钙化的中层血管病变(也称为Mönckeberg's中层钙化性硬化症)、中层钙化、弹性蛋白沉积、钙化尿毒症性动脉病变，钙化性主动脉瓣膜狭窄和/或门静脉钙化。在某些实施方式中，所述与钙化的动脉粥样硬化相关的疾病是动脉粥样硬化、高脂血症、骨质疏松症、高血压、炎症、2型糖尿病、终末期肾病、所需截肢、弹性假黄瘤、高脂血症、先天性二尖瓣、风湿性心脏病和/或肝脏疾病。在某些实施方式中，根据本文提供的方法治疗的对象患有CKD诱导的心脏重量提高。在某些实施方式中，所述对象患有心脏肥大。在某些实施方式中，所述对象患有肌细胞过度生长。在某些实施方式中，所述对象患有提高的动脉和/或血管硬化。在某些实施方式中，所述对象患有LVH。

[0176] 在某些实施方式中，根据本文描述的方法治疗的对象与如章节8.6中描述的参考群体相比具有不希望的高水平的主动脉钙、血管钙化、动脉硬化水平升高和/或成骨细胞数量。在某些实施方式中，根据本文描述的方法治疗的对象与如章节8.6中描述的参考群体相比具有发生不希望的高水平的主动脉钙、血管钙化、动脉硬化水平升高和/或成骨细胞数量的风险，例如，患有慢性肾病的对象。在某些实施方式中，根据本文提供的方法治疗的对象与如章节8.6中描述的参考群体相比具有主动脉粥样斑中的钙沉积。在某些实施方式中，根据本文提供的方法治疗的对象与如章节8.6中描述的参考群体相比具有主动脉硬化形式的动脉硬化水平升高。在某些实施方式中，根据本文提供的方法治疗的对象与如章节8.6中描述的参考群体相比具有提高高血压的动脉硬化水平升高水平。

[0177] 在某些实施方式中，根据本文提供的方法治疗的对象患有与肾病相关和/或由肾病引起的心血管疾病。在某些实施方式中，所述肾病是慢性肾病。在某些实施方式中，根据本文提供的方法治疗的对象患有慢性肾病。在某些实施方式中，所述对象患有继发于慢性肾病的心血管疾病。在某些实施方式中，所述慢性肾病达到3期、4期、5期或5D期。在具体的实施方式中，所述肾病是终末期肾病。在某些实施方式中，所述对象具有成年人的小于60ml/分钟/1.73m^{2}或儿童对象的小于89ml/分钟/1.73m^{2}的肾小球过滤率。参见Moe et al., 2006,Kidney International 69:1945-1953。在某些实施方式中，所述对象是成年人，其中

所述对象具有小于50ml/分钟/1.73m^2、40ml/分钟/1.73m^2、30ml/分钟/1.73m^2、20ml/分钟/1.73m^{2}或小于10ml/分钟/1.73m^{2}的肾小球过滤率。在某些实施方式中，所述对象是儿童对象，其中所述儿童对象具有小于80ml/分钟/1.73m^2、70ml/分钟/1.73m^2、60ml/分钟/1.73m^2、50ml/分钟/1.73m^2、40ml/分钟/1.73m^2、30ml/分钟/1.73m^2、20ml/分钟/1.73m^{2}或小于10ml/分钟/1.73m^{2}的肾小球过滤率。不受理论的限制，在成年人对象中小于60ml/分钟/1.73m^{2}以及在儿童对象中小于89ml/分钟/1.73m^{2}的肾小球过滤率引起钙水平、磷水平、PTH水平和维生素D新陈代谢方面可检测的异常；这些标志物的异常水平引起骨骼疾病。在某些实施方式中，所述对象具有参考群体降低的菊糖清除率，例如，如章节8.6中描述的参考群体。在某些实施方式中，所述对象具有参考群体降低的血尿氮水平，例如，如章节8.6中描述的参考群体。在某些实施方式中，所述对象是高磷酸盐血的。在某些实施方式中，所述对象患有肾脏纤维化。

[0178] 在某些实施方式中，所述对象患有Alport's综合征。在某些实施方式中，所述对象在与Alport's综合征相关的COL4A5基因中有一个或更多个体细胞突变。

[0179] 在某些实施方式中，根据本文提供的方法治疗的对象具有与慢性肾病相关的骨骼病理，即，CKD-矿物质/骨骼失调(CKD-MBD)。参见Moe et al.,2006,Kidney International 69:1945-1953。在某些实施方式中，与参考群体，例如，如章节8.6中描述的参考群体相比，所述对象具有降低的小梁骨体积。在某些实施方式中，与参考群体，例如，如章节8.6中描述的参考群体的小梁厚度相比，所述对象具有降低的小梁厚度。在某些实施方式中，与参考群体，例如，如章节8.6中描述的参考群体的侵蚀面骨表面比相比，所述对象具有提高的侵蚀面骨骼表面比。在某些实施方式中，与参考群体，例如，如章节8.6中描述的参考群体的破骨细胞水平相比，所述对象具有提高的破骨细胞水平。在某些实施方式中，与参考群体，例如，如章节8.6中描述的参考群体的成骨细胞表面骨骼表面比相比，所述对象具有提高的成骨细胞表面骨骼表面比。在某些实施方式中，与参考群体，例如，如章节8.6中描述的参考群体的成骨细胞水平相比，所述对象具有提高的成骨细胞水平。在某些实施方式中，与参考群体中，例如，如章节8.6中描述的参考群体的骨形成率相比，所述对象具有降低的骨形成率。在某些实施方式中，所述CKD-MBD由肾性骨营养不良和血管钙化组成。在某些实施方式中，所述CKD-MBD由肾性骨营养不良组成。在某些实施方式中，所述CKD-MBD是低周转CKD-MBD。在某些实施方式中，所述CKD-MBD由动脉硬化水平升高和/或LVH组成。低周转CKD-MBD可以通过以下的表1中列出的组织学特征来诊断。参见National Kidney Foundation,Kidney Disease Outcomes Quality Initiative Guidelines at the website of the National Kidney Foundation。在某些实施方式中，所述对象具有高周转ROD。

[0180] 表1.低周转CKD-MBD的组织学特征

[0181]

特征	衰弱的	骨软化
骨形成		
小梁骨体积	正常，低	可变量 低、正常或高
类骨质体积	正常，低	高-非常高
类骨质接缝厚度	正常，低	高-非常高
成骨细胞数量	低	低
骨形成率	低-非常低	低-非常低
矿化滞后时间	正常	延长
骨吸收		
受腐蚀骨周长	正常，低	可变量 经常低，可以高
破骨细胞数量	低	低、可以正常或高
骨髓纤维化	没有	没有

[0182] 在某些实施方式中，所述对象正经历血液透析。

[0183] 在某些实施方式中，根据本文描述的方法治疗的对象患有终末期肾病。在某些实施方式中，根据本文描述的方法治疗的对象经历透析。

[0184] 在某些实施方式中，根据本文描述的方法治疗的对象具有与参考群体中的骨吸收相比提高的骨吸收。在某些实施方式中，所述提高的骨吸收通过CTX的水平确定。在某些实施方式中，所述CTX是害怕如章节8.6中描述的确定。在某些实施方式中，所述骨吸收如章节8.6中描述的评估。

[0185] 在某些实施方式中，根据本文描述的方法治疗的对象可以是任何年龄。在某些实施方式中，根据本文描述的方法治疗的对象是小于18岁。在具体的实施方式中，根据本文描述的方法治疗的对象是小于13岁。在另一个具体的实施方式中，根据本文描述的方法治疗的对象是小于12岁、小于11岁、小于10岁、小于9岁、小于8岁、小于7岁、小于6岁或小于5岁。在另一个具体的实施方式中，根据本文描述的方法治疗的对象是1-3岁、3-5岁、5-7岁、7-9岁、9-11岁、11-13岁、13-15岁、15-20岁、20-25岁、25-30岁或大于30岁。在另一个具体的实施方式中，根据本文描述的方法治疗的对象是30-35岁、35-40岁、40-45岁、45-50岁、50-55岁、55-60岁或大于60岁。在另一个具体的实施方式中，根据本文描述的方法治疗的对象是60-65岁、65-70岁、70-75岁、75-80岁或大于80岁。

[0186] 在某些实施方式中，根据本文提高的方法治疗的对象患有终末期肾病。在某些实施方式中，根据本文提供的方法治疗的对象在血液透析。在某些实施方式中，根据本文提供的方法治疗的对象早先施用了促红细胞生成素刺激剂。在某些实施方式中，根据本文提供的方法治疗的对象患有终末期肾病，在血液透析，并且早先施用了促红细胞生成素刺激剂。

[0187] 本领域技术人员将认识到，Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2、Sm22-alpha、

phosphosmad3和尿蛋白的水平和/或活性可以分别与相应参考群体中的水平和/或活性独立比较，例如，如上文在章节8.3.1中描述的。

[0188] 8.5 ACTRII信号转导抑制剂

[0189] 本节中描述的和本领域已知的ActRII信号转导抑制剂可以用于本文提供的方法。在某些实施方式中，本节描述的ActRII信号转导抑制剂可以用于本文提供的方法中(参见，章节8.3)。

[0190] 本文涵盖的ActRII信号转导抑制剂包括ActRIIA信号转导抑制剂和ActRIIB信号转导抑制剂(见下文)。在某些实施方式中，ActRII信号转导抑制剂是特异于ActRIIA信号转导的。在其他实施方式中，ActRII信号转导抑制剂是特异于ActRIIB信号转导的。在某些实施方式中，ActRII信号转导抑制剂优先地抑制ActRIIA信号转导。在其他实施方式中，ActRII信号转导抑制剂优先地抑制ActRIIB信号转导。在某些实施方式中，ActRII信号转导抑制剂抑制ActRIIA信号转导和ActRIIB信号转导两者。

[0191] 在某些实施方式中，ActRII信号转导抑制剂可以是包含ActRII的激活素结合结构域的多肽。不受理论的限制，这种包含激活素结合结构域的多头隔离了激活素，从而阻止激活素信号转导。这些包含激活素结合结构域的多肽可以包含ActRII的细胞外结构域的全部或一部分(即，ActRIIA的全部或部分细胞外结构域，或ActRIIB的全部或部分细胞外结构域)。在具体的实施方式中，ActRII的细胞外结构域是可溶的。

[0192] 在某些实施方式中，包含激活素结合结构域的多肽连接到抗体的Fc部分(即，产生包含ActRII受体的包含激活素结合结构域的多肽与抗体的Fc部分的轭合物)。不受理论的限制，抗体部分为所述轭合物赋予了提高的稳定性。在某些实施方式中，激活素结合结构域通过接头，例如肽接头连接到抗体的Fc部分。

[0193] 本文描述的组合物和方法中使用的ActRII信号转导抑制剂包含直接地或间接地、细胞外地或细胞内地抑制ActRIIA信号转导和/或ActRIIB信号转导的分子。在某些实施方式中，本文描述的组合物和方法中使用的ActRIIA信号转导和/或ActRIIB信号转导的抑制剂通过与其本身的受体相互作用来抑制ActRIIA信号转导和/或ActRIIB信号转导。在其他实施方式中，本文描述的组合物和方法中使用的ActRIIA信号转导和/或ActRIIB信号转导的抑制剂通过与ActRIIA和/或ActRIIB配体，例如激活素的相互作用抑制ActRIIA信号转导和/或ActRIIB信号转导。

[0194] 8.5.1 ACTRIIA信号转导的抑制剂

[0195] 如本文使用的，术语“ActRIIA”是指来自任何物种的激活素IIA型受体(ActRIIA)蛋白的家族，以及通过诱变或其他修饰衍生自这些ActRIIA蛋白的变体。本文提及ActRIIA被理解为是提及当前鉴定的形式的任一种。ActRIIA家族的成员一般是跨膜的蛋白质，由具有富半胱氨酸区的配体结合细胞外结构域、跨膜结构域、以及具有预测的丝氨酸/苏氨酸激酶活性的细胞质结构域组成。

[0196] 本文描述的组合物和方法中使用的ActRIIA信号转导抑制剂非限制性地包括，结合激活素的可溶的ActRIIA多肽；结合激活素(特别是激活素A或B亚基，还成为β_A或β_B)并破坏ActRIIA结合的抗体；结合ActRIIA并破坏激活素结合的抗体；对于激活素或ActRIIA结合而选择的非抗体蛋白质(参见，例如WO/2002/088171，WO/2006/055689，WO/2002/032925，WO/2005/037989，US 2003/0133939，和US 2005/0238646，它们的每一个通过完全引用合并

在本文中，作为这类蛋白质和设计与选择这类蛋白质的实例)；为激活素或ActRIIA结合而选择的随机肽，它们可以轭合到Fc结构域。

[0197] 在某些实施方式中，具有激活素或ActRIIA结合活性的两种或更多种不同的蛋白质(或其他部分)，特别是分别阻断I型(例如，可溶的I型激活素受体)和II型(例如，可溶的II型激活素受体)结合位点的激活素结合物可以连接在一起，产生抑制ActRIIA信号转导的双功能或多功能结合分子，因而可以用于本文描述的组合物和方法。在某些实施方式中，抑制ActRIIA信号转导的激活素-ActRIIA信号转导轴拮抗剂包括核酸适体、小分子和其他试剂，用于本文描述的组合物和方法中。

[0198] 8.5.1.1 包含ActRIIA多肽的ActRIIA信号转导抑制剂

[0199] 术语“ActRIIA多肽”包括这些多肽，其包含ActRIIA家族成员的任何天然发生的多肽以及保持了有用活性的其任何变体(包括突变体、片段、融合物和肽模拟物形式)。例如，ActRIIA多肽包括一些多肽，其衍生自任何已知的ActRIIA的序列、具有至少约80％相同于ActRIIA多肽的序列的序列，任选地至少85％、90％、95％、97％、98％、99％或更高的同一性。例如，ActRIIA多肽可以结合并抑制ActRIIA蛋白质和/或激活素的功能。可以针对其促进骨骼生长和骨矿化的能力选择ActRIIB多肽。ActRIIA多肽的实例包括人ActRIIA前体多肽(SEQ ID NO:1)和可溶的人ActRIIA多肽(例如，SEQ ID NO:2、3、7和12)。对于氨基酸序列在SEQ ID NO:1中描述的ActRIIA前体多肽，人ActRIIA前体多肽的信号肽位于氨基酸位置1到20；细胞外结构域位于氨基酸位置21到135，人ActRIIA前体多肽(SEQ ID NO:1)的N-连接的糖基化位点位于SEQ ID NO:1的氨基酸位置43和56。编码SEQ ID NO:1的人ActRIIB前体多肽的核酸序列被公开为SEQ ID NO:4(Genbank条目NM_001616的核苷酸164-1705)。编码SEQ ID NO:2的可溶的人ActRIIA多肽的核酸序列被公开为SEQ ID NO:5。序列的描述参见表21。

[0200] 在具体的实施方式中，本文描述的组合物和方法中使用的ActRIIA多肽是可溶的ActRIIA多肽。ActRIIA蛋白的细胞外结构域可以结合激活素，一般是可溶的，因而可以称为可溶的激活素结合ActRIIA多肽。因而，如本文使用的，术语“可溶的ActRIIA多肽”一般是指包含ActRIIA蛋白的细胞外结构域的多肽，包括ActRIIA蛋白的任何天然发生的细胞外结构域以及其任何变体(包括突变体、片段、肽模拟物形式)。可溶的ActRIIA多肽可以结合激活素；然而，野生型ActRIIA蛋白在结合激活素与GDF8/11方面不展现显著的选择性。天然的或改变的ActRIIA蛋白可以添加对激活素的特异性，通过将它们与第二激活素选择结合试剂偶联。可溶的激活素结合ActRIIA多肽的实例包括SEQ ID NO:2、3、7、12和13中例举的可溶多肽。可溶的激活素结合ActRIIA多肽的其他实例除了ActRIIA蛋白的细胞外结构域之外包括信号序列，例如，蜜蜂蜂毒前导序列(SEQ ID NO:8)、组织纤溶酶原激活物(TPA)前导区(SEQ ID NO:9)或天然ActRIIA前导区(SEQ ID NO:10)。SEQ ID NO:13中例举的ActRIIA-hFc多肽使用TPA前导区。

[0201] 在某些实施方式中，本文描述的组合物和方法中使用的ActRIIA信号转导的抑制剂包含轭合物/融合蛋白，其包含与抗体的Fc部分连接的ActRIIA的激活素结合结构域。在某些实施方式中，激活素结合结构域通过接头，例如肽接头连接到抗体的Fc部分。任选地，所述Fc结构域在残基例如Asp-265、赖氨酸322和Asn-434处具有一个或更多个突变。在某些情况下，具有一个或更多个这些突变(例如，Asp-265突变)的突变Fc结构域相对于野生型Fc

结构域具有降低的结合Fcγ受体的能力。在其它情况下，具有一个或更多个这些突变(例如，Asn-434突变)的突变Fc结构域相对于野生型Fc结构域具有提高的结合MHC I类相关Fc受体(FcRN)的能力。包含与Fc结构域融合的ActRIIA的可溶细胞外结构域的示范性的融合蛋白在SEQ ID NO:6、7、12和13中列出。

[0202] 在具体的实施方式中，本文描述的组合物和方法中使用的ActRIIA信号转导抑制剂包含与抗体的Fc部分连接的ActRIIA的细胞外结构域，或其部分，其中所述ActRIIA信号转导抑制剂包含至少75％相同于选自SEQ ID NO:6、7、12和13的氨基酸序列的氨基酸序列。在另一个具体的实施方式中，本文描述的组合物和方法中使用的ActRIIA信号转导抑制剂包含与抗体的Fc部分连接的ActRIIA的细胞外结构域，或其部分，其中所述ActRIIA信号转导抑制剂包含至少80％、85％、90％、95％、96％、97％、98％或99％相同于选自SEQ ID NO:6、7、12和13的氨基酸序列的氨基酸序列。

[0203] 在某些实施方式中，本文描述的组合物和方法中使用的ActRIIA信号转导的抑制剂包含ActRIIA的细胞外结构域的截短的形式。所述截短可以是在ActRIIA多肽的羧基末端和/或氨基末端。在某些实施方式中，相对于成熟的ActRIIB多肽细胞外结构域，所述截短可以是1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25个氨基酸长度。在某些实施方式中，所述截短可以是1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25个成熟ActRIIA多肽细胞外结构域的N-末端氨基酸。在某些实施方式中，所述截短可以是1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25个成熟ActRIIA多肽细胞外结构域的C-末端氨基酸。例如，ActRIIA的截短形式包括具有氨基酸20-119；20-128；20-129；20-130；20-131；20-132；20-133；20-134；20-131；21-131；22-131；23-131；24-131；和25-131的多肽，其中所述氨基酸位置参考SEQ ID NO:1中的氨基酸位置。

[0204] 在某些实施方式中，本文描述的组合物和方法中使用的ActRIIA信号转导的抑制剂包含具有一个或更多个氨基酸取代的ActRIIA的细胞外结构域。在某些实施方式中，本文描述的组合物和方法中使用的ActRIIA信号转导的抑制剂包含还带有氨基酸取代的ActRIIA细胞外结构域的截短的形式。

[0205] 在具体的实施方式中，用于本文描述的组合物和方法的ActRIIA信号转导抑制剂是人ActRIIA受体的细胞外结构域与IgG1的Fc部分之间的融合蛋白。在另一个具体的实施方式中，用于本文描述的组合物和方法的ActRIIA信号转导抑制剂是人ActRIIA受体的截短的细胞外结构域与IgG1的Fc部分之间的融合蛋白。在另一个具体的实施方式中，用于本文描述的组合物和方法的ActRIIA信号转导抑制剂是人ActRIIA受体的截短的细胞外结构域与IgG1的Fc部分之间的融合蛋白，其中所述人ActRIIA受体的截短的细胞外结构域具有一个或更多个氨基酸取代。

[0206] 例如，通过筛选重组地产自编码ActRIIA多肽的核酸的相应片段的多肽，可以获得ActRIIA多肽的功能活性片段。此外，片段可以使用本领域已知的技术例如，常规的Merrifield固相f-Moc或t-Boc化学作用来化学地合成。所述片段可以被产生(重组地或通过化学合成)和测试，来鉴定可以用作ActRIIA蛋白的或激活素介导的信号转导的拮抗剂(抑制剂)的那些肽片段。

[0207] 此外，例如，通过筛选重组地产自编码ActRIIA多肽的相应诱变核酸的修饰多肽的

文库，可以获得ActRIIA多肽的功能活性的变体。所述变体可以被产生和测试，来鉴定可以用作ActRIIA蛋白的或激活素介导的信号转导的拮抗剂(抑制剂)的那些。在某些实施方式中，ActRIIA多肽的功能变体包含至少75％相同于选自SEQ ID NO:2或3的氨基酸序列的氨基酸序列。在某些情况下，所述功能变体具有至少80％、85％、90％、95％、97％、98％、99％或100％相同于选自SEQ ID NO:2或3的氨基酸序列的氨基酸序列。

[0208] 例如，通过为了诸如增强效力或稳定性(例如，体外货架寿命或对体内蛋白水解降解的抗性)的目的修饰ActRIIA多肽的结构，可以产生功能变体。当被选择以保持激活素结合时，这种修饰的ActRIIA多肽可以被认为是天然发生的ActRIIA多肽的功能等效物。例如，通过氨基酸取代、删除或添加，也可以产生修饰的ActRIIA多肽。例如，合理的是预计，亮氨酸用异亮氨酸或缬氨酸、天冬氨酸用谷氨酸、苏氨酸用丝氨酸的独立替换，或氨基酸用结构相关氨基酸的相似性替换(例如，保守突变)将不会对产生的分子的生物学活性有重大的影响。保守性替换是在其侧链相关的氨基酸家族内发生的替换。通过评估变体ActRIIA多肽在细胞中以类似于野生型ActRIIA多肽的方式产生反应的能力，ActRIIA多肽的氨基酸序列改变是否产生功能性同源物可以容易地测定。

[0209] 在某些实施方式中，在本文提供的描述的组合物和方法中使用的ActRIIA信号转导抑制剂可以包含ActRIIA多肽，所述ActRIIA多肽具有可以改变所述多肽的糖基化的一个或更多个特定突变。这样的突变可以引入或除去一个或更多个糖基化位点，例如，O-连接的或N-连接的糖基化位点。天冬酰胺连接的糖基化识别位点一般包含三肽序列，天冬酰胺-X-苏氨酸(或天冬酰胺-X-丝氨酸)(其中“X”是任意氨基酸)，其被适当的细胞糖基化酶特异性地识别。也可以通过向野生型ActRIIA多肽的序列中添加或取代一个或多个丝氨酸或苏氨酸残基(用于O－连接的糖基化位点)来进行改变。在糖基化识别位点的第一或第三个氨基酸位置的一个或两个上的各种氨基酸取代或删除(和/或在第二位置上的氨基酸删除)引起所述修饰的三肽序列处的非糖基化。提高ActRIIA多肽上碳水化合物部分的数量的另一种方式是通过糖苷对ActRIIA多肽的化学或酶学偶联。取决于使用的偶联方式，糖可以附着于(a)精氨酸和组氨酸；(b)游离羧基基团；(c)游离巯基基团，例如半胱氨酸的；(d)游离羧基基团，例如，丝氨酸、苏氨酸或羟脯氨酸的；(e)芳香族残基，例如苯丙氨酸、酪氨酸或色氨酸的；或(f)谷氨酰胺的酰胺基团。这些方法在1987年9月11日公开的WO87/05330中，以及在Aplin and Wriston(1981)CRC Crit.Rev.Biochem.,pp.259-306中描述了，通过引用合并在本文中。除去ActRIIA多肽上存在的一个或更多个碳水化合物部分可以化学地或酶学地实现。化学的脱糖基化可能涉及例如将ActRIIA多肽暴露于化合物三氟甲磺酸或相当的化合物。这种处理引起除了连接的糖(N-乙酰葡萄糖胺或N-乙酰半乳糖胺)之外大部分或所有的糖的裂解，而保留氨基酸序列完整。化学的脱糖基化由Hakimuddin et al.(1987)Arch.Biochem.Biophys.259:52和Edge et al.(1981)Anal.Biochem.118:131进一步描述了。ActRIIA多肽上碳水化合物部分的酶裂解可以通过使用多种内切和外切糖苷酶来实现，如Thotakura et al.(1987)Meth.Enzymol.138:350所描述的。ActRIIA多肽的序列可以被调整，视情况，取决于使用的表达系统的类型，因为哺乳动物、酵母、昆虫和植物细胞可能都引入不同的糖基化模式，其可能受到肽的氨基酸序列的影响。一般地，用于人类的ActRIIA蛋白可以在提供适当的糖基化的哺乳动物细胞系中表达，例如，HEK293或CHO细胞系，而其他表达系统，例如其他哺乳动物表达细胞系、具有工程化的糖基化酶的酵母细胞系以及昆

虫细胞预计也是有用的。

[0210] 本文进一步提供的是产生突变体的方法，特别是ActRIIA多肽的组合突变体的集合，以及截短突变体；组合突变体的库对于鉴定功能性变体序列是特别有用的。筛选这种组合文库的目的可以是产生，例如，可以用作激动剂或拮抗剂的ActRIIA多肽变体，或作为选择，其一起具有新的活性。下文提供了多种筛选分析，这样的分析可以用于评估变体。例如，可以筛选ActRIIA多肽变体结合ActRIIA配体，防止ActRIIA配体与ActRIIA多肽结合，或干扰由ActRIIA配体引起的信号转导的能力。

[0211] 可以产生组合衍生的变体，其相对于天然发生的ActRIIA多肽具有选择的或一般提高的效价。同样，诱变可导致细胞内半衰期与相应的野生型ActRIIA多肽显著不同的变体。例如，改变的蛋白可以变得对引起天然ActRIIA多肽的破坏或灭活的蛋白水解降解或其他细胞代谢过程更稳定或更不稳定。这样的变体和编码它们的基因可以被利用通过调节ActRIIA多肽的半衰期来改变ActRIIA多肽水平。例如，短的半衰期可以产生更短暂的生物学效果，可以容许对象体内重组ActRIIA多肽水平的紧密的控制。在Fc融合蛋白中，突变可以在接头(如果有的话)和/或Fc部分进行，来改变蛋白质的半衰期。

[0212] 通过编码多肽文库的简并基因文库的方式可以产生组合文库，所述多肽文库各自包括可能的ActRIIA多肽序列的至少一部分。例如，合成的寡核苷酸的混合物可以酶学连接到基因序列中，从而可能的ActRIIA多肽核苷酸序列的简并集合可以作为单独的多肽表达，或作为选择，作为更大的融合蛋白的集合(例如，用于噬菌体展示)。

[0213] 有许多方法可以从简并寡核苷酸序列中产生可能的同源物的文库。可以在自动DNA合成仪中进行简并基因序列的化学合成，合成的基因然后可以连接到适合的载体中用于表达。简并寡核苷酸的合成是本领域公知的(参见，例如Narang,S A(1983)Tetrahedron 39:3;Itakura et al.,(1981)Recombinant DNA,Proc.3rd Cleveland Sympos.Macromolecules,ed.AG Walton,Amsterdam:Elsevier pp 273-289;Itakura et al.,(1984)Annu.Rev.Biochem.53:323;Itakura et al.,(1984)Science 198:1056;Ike et al.,(1983)Nucleic Acid Res.11:477)。这样的技术已经用于其他蛋白质的定向进化(参见，例如Scott et al.,(1990)Science 249:386-390;Roberts et al.,(1992)PNAS USA 89:2429-2433;Devlin et al.,(1990)Science 249:404-406;Cwirla et al.,(1990)PNAS USA 87:6378-6382;以及美国专利Nos.5,223,409,5,198,346和5,096,815)。

[0214] 做为选择，可以利用其他形式的诱变来产生组合文库。例如，ActRIIA多肽变体可以通过筛选来从文库中产生和分离，利用，例如，丙氨酸扫描诱变等(Ruf et al.,(1994)Biochemistry 33:1565-1572;Wang et al.,(1994)J.Biol.Chem.269:3095-3099;Balint et al.,(1993)Gene 137:109-118;Grodberg et al.,(1993)Eur.J.Biochem.218:597-601;Nagashima et al.,(1993)J.Biol.Chem.268:2888-2892;Lowman et al.,(1991)Biochemistry 30:10832-10838;和Cunningham et al.,(1989)Science 244:1081-1085)，通过接头扫描诱变(Gustin et al.,(1993)Virology 193:653-660;Brown et al.,(1992)Mol.Cell Biol.12:2644-2652;McKnight et al.,(1982)Science 232:316);通过饱和诱变(Meyers et al.,(1986)Science 232:613);通过PCR诱变(Leung et al.,(1989)Method Cell Mol Biol 1:11-19);或通过随机诱变，包括化学诱变等(Miller et al.,(1992)A Short Course in Bacterial Genetics,CSHL Press,Cold Spring Harbor,N.Y.;和

Greener et al.,(1994)Strategies in Mol Biol 7:32-34)。接头扫描诱变,特别是在组合场景中,是鉴定ActRIIA多肽的截短形式(生物活性)的吸引人的方法。

[0215] 大量的技术是本领域已知的,用于筛选由点突变或截短产生的组合库的基因产物,就此而言,用于筛选cDNA库中具有某些性质的基因产物。这些技术一般将适合于通过ActRIIA多肽的组合诱变产生的基因库的快速筛选。用于筛选大的基因文库的最广泛使用的技术一般包括将基因文库克隆到可复制的表达载体中,用产生的载体库转化合适的细胞,并在一定条件下表达组合的基因,在所述条件中期望的活性的检测便于相对容易地分离编码被检出产物的基因的载体。优选的分析包括激活素结合分析和激活素介导的细胞信号转导分析。

[0216] 在某些实施方式中,本文描述的方法和组合物的抑制剂中使用的ActRIIA多肽可以进一步包含翻译后修饰,除了天然存在于所述ActRIIA多肽中的之外。这种的修饰可以包括但不限于,乙酰化、羧基化、糖基化、磷酸化、脂质化和酰化。结果,修饰的ActRIIA多肽可以含有非氨基酸元件,例如,聚乙二醇、脂质、聚糖或单体和磷酸盐。这种非氨基酸元件对ActRIIA多肽的功能的影响可以通过熟练技术人员已知的任何方法来测试。当通过裂解ActRIIA多肽的新生形式来在细胞中产生ActRIIA多肽时,翻译后的加工对于蛋白质的正确折叠和/或功能可能也是重要的。不同的细胞(例如CHO、HeLa、MDCK、293、W138、NIH-3T3或HEK293)具有这种翻译后活性的特定细胞机制和特征性的机制,可以选择来确保ActRIIA多肽的正确修饰和加工。

[0217] 在某些方面,本文描述的方法和组合物的抑制剂中使用的ActRIIA多肽的功能变体或修饰形式包括具有至少一部分ActRIIA多肽和一个或更多个融合结构域的融合蛋白。这样的融合结构域的公知的实例包括但不限于,多组氨酸、Glu-Glu、谷胱甘肽S转移酶(GST)、硫氧还蛋白、蛋白A、蛋白G、免疫球蛋白重链恒定区(Fc)、麦芽糖结合蛋白(MBP)或人血清白蛋白。可以选择融合结构域以赋予期望的性质。例如,某些融合结构域对于通过亲和层析分离融合蛋白是特别有用的。对于亲和纯化来说,使用亲和层析的相关基质,例如,谷胱甘肽-、淀粉酶-和镍-或钴-钶合的树脂。许多这样的基质是以“试剂盒”形式可获得的,例如,对于(HIS6)融合配体有用的Pharmacia GST纯化系统,以及QIAexpress.TM.系统(Qiagen)。作为另一个实例,可以选择融合结构域以促进ActRIIA多肽的检测。这样的检测结构域的实例包括各种荧光蛋白(例如,GFP),以及“表位标签”,其通常是有特异性抗体的短肽序列。特异性单克隆抗体容易获得的公知的表位标签包括FLAG、流感病毒血凝素(HA)和c-myc标签。在某些情况下,融合结构域具有蛋白酶裂解位点,例如,因子Xa或凝血酶的,其容许相关蛋白酶部分地消化所述融合蛋白,从而由此释放重组蛋白质。释放的蛋白质然后可以通过随后的层析分离与融合结构域分离。在某些优选的实施方式中,ActRIIA多肽与使所述ActRIIA多肽在体内稳定化的结构域融合(“稳定物”结构域)。“稳定”是指提高血清半衰期的任何事,不论是由于降低的破坏、降低的肾脏清除、还是其他的药物动力学效应。已知与免疫球蛋白的Fc部分融合为大范围的蛋白质赋予期望的药物动力学性质。同样地,与人血清白蛋白融合可以赋予期望的性质。可以选择的其他类型的融合结构域包括多聚化(例如,二聚化、四聚化)结构域和功能结构域(赋予其他生物学功能的,例如,进一步刺激骨骼生长或肌肉生长,如所希望的)。

[0218] 要理解的是融合蛋白的不同元件可以以与期望的功能一致的任何方式布置。例

如，ActRIIA多肽可以置于异源结构域的C-末端，或做为选择，异源结构域可以置于ActRIIA多肽的C-末端。ActRIIA多肽结构域和异源结构域不必在融合蛋白中相邻，其他的结构域或氨基酸序列可以被包括在任一结构域的C-末端或N-末端，或两个结构域之间。

[0219] 在某些实施方式中，本文描述的方法和组合物的抑制剂中使用的ActRIIA多肽可以含有能够稳定化所述ActRIIA多肽的一个或更多个修饰。例如，这样的修饰可以增强ActRIIA多肽的体外半衰期，增强ActRIIA多肽的循环半衰期，或降低ActRIIA多肽的蛋白水解酶降解。这样的稳定化修饰可以包括，但不限于，融合蛋白(包括，例如，包含ActRIIA多肽和稳定化结构域的融合蛋白)、糖基化位点的修饰(包括，例如，向ActRIIA多肽添加糖基化位点)，以及碳水化合物部分的修饰(包括，例如，从ActRIIA多肽除去碳水化合物部分)。对于融合蛋白来说，ActRIIA多肽融合到稳定物结构域，例如IgG分子(例如，Fc结构域)。如本文使用的，术语“稳定物结构域”不仅是指融合蛋白情况下的融合结构域(例如，Fc)，还包括非蛋白性的修饰，例如，碳水化合物部分，或非蛋白性的聚合物，例如，聚乙二醇。

[0220] 在某些实施方式中，分离自或基本上没有其他蛋白质的ActRIIA多肽的分离的和/或纯化的形式可以用于本文描述的方法和组合物。ActRIIA多肽一般可以通过从重组核酸表达来产生。

[0221] 在某些方面，本文描述的组合物和方法中使用的ActRIIA多肽使用分离的和/或重组的核酸产生，所述核酸编码任一种ActRIIA多肽(例如，可溶的ActRIIA多肽)，包括本文公开的片段、功能变体和融合蛋白。例如，是4编码天然发生的人ActRIIA前体多肽，而SEQ ID NO:5编码ActRIIA的加工的细胞外结构域。这样的核酸可以是单链的或是双链的。所述核酸可以是DNA或RNA分子。这些核酸可以用于，例如，制造ActRIIA多肽的方法，或作为直接的治疗试剂(例如，在基因治疗方法中)。

[0222] 在某些方面，编码ActRIIA多肽的核酸可以包括作为SEQ ID NO:4或5的变体的核酸。变体核苷酸序列包括差异在于一个或更多个核苷酸取代、添加或删除的序列，例如，等位变体。

[0223] 在某些实施方式中，编码ActRIIA多肽的分离的或重组的核酸序列可以是至少80％、85％、90％、95％、97％、98％、99％或100％相同于SEQ ID NO:4或5。本领域普通技术人员将理解，与SEQ ID NO:4或5互补的核酸序列以及SEQ ID NO:4或5的变体可以用于生产适用于本文描述的方法和组合物的ActRIIA多肽。在进一步的实施方式中，这样的核酸序列可以是分离的，重组的和/或融合到异源核苷酸序列的，或是来自DNA文库。

[0224] 在其他实施方式中，用于生产适用于本文描述的方法和组合物的ActRIIA多肽的核酸可以包括在高度严格条件下与SEQ ID NO:4或5指定的核苷酸序列、SEQ ID NO:4或5的互补序列或其片段杂交的核苷酸序列。本领域普通技术人员将理解，促进DNA杂交的合适的严格条件可以是变化的。例如，人们可以在6.0倍氯化钠/柠檬酸钠(SSC)、大约45℃下杂交，最后2.0倍SSC在50℃下洗涤。例如，洗涤步骤中的盐浓度可以选自50℃下的约2.0倍SSC的低严格度，到50℃下约0.2倍SSC的高严格度。此外，洗涤步骤中的温度可以从室温约22℃下的低严格条件，提高到大约65℃的高严格条件。温度和盐度可以都变化，或者温度或盐浓度保持不变而另一个变量发生改变。在一个实施方式中，在6倍SSC室温下的低严格条件下杂交、随后在室温下2倍SSC洗涤的核酸可以用于本文描述的方法和组合物。

[0225] 由于遗传密码的简并性而不同于SEQ ID NO:4或5中所列核酸的分离的核酸也可

以用于生产适用于本文描述的方法和组合物的ActRIIA多肽。例如，许多氨基酸由超过一种三联体指定。指定相同氨基酸的密码子，或同义密码子(例如，CAU和CAC对于组氨酸是同义的)可以产生“沉默”突变，其不影响蛋白质的氨基酸序列。然而，预计的是，引起目标蛋白质的氨基酸序列改变的DNA序列多态性将存在于哺乳动物细胞。本领域技术人员将理解，编码特定蛋白质的核酸的一个或更多个核苷酸(达到核苷酸的约3-5％)中的这些变异由于天然的等位变异可以存在于给定物种的个体中。

[0226] 在某些实施方式中，重组核酸可以可操作连接到表达构建体中的一个或更多个调节核苷酸序列。调节核苷酸序列一般将适合于用来表达的宿主细胞。许多类型的适当的表达载体和适合的调节序列是本领域已知的，用于多种宿主细胞。一般地，所述一个或更多个调节核苷酸序列可以包括，但不限于，启动子序列、前导区或信号序列、核糖体结合位点、转录起始和终止序列、翻译起始和终止序列，以及增强子或激活物序列。本领域已知的组成型或可诱导启动子是本文期待的。启动子可以是天然发生的启动子，或组合了超过一种启动子的元件的杂合启动子。表达构建体可以存在于细胞中或游离体例如质粒上，或者表达构建体可以插入染色体中。在优选的实施方式中，表达载体含有选择性标记基因以容许选择转化的宿主细胞。选择性标记基因是本领域公知的，将随使用的宿主细胞而变化。

[0227] 在某些方面，适用于本文描述的方法和组合物的ActRIIA多肽的生产中使用的核酸可以在表达载体中提供，所述表达载体包含编码ActRIIA多肽的核苷酸序列并与至少一个调节序列可操作连接。调节序列是公知的，被选择来指导ActRIIA多肽的表达。因而，术语调节序列包括启动子、增强子和其他表达控制元件。示范性的调节序列在Goeddel;Gene Expression Technology:Methods in Enzymology,Academic Press,San Diego,Calif.(1990)中描述。例如，控制与之可操作地连接的DNA序列的表达的各种表达控制序列的任一种，可以用于这些载体来表达编码ActRIIA多肽的DNA序列。这类有用的表达控制序列包括，例如，SV40的早期和晚期启动子、tet启动子、腺病毒或巨细胞病毒即时早期启动子、RSV启动子、lac系统、trp系统、TAC或TRC系统、其表达由T7RNA聚合酶指导的T7启动，噬菌体λ的主要操纵子和启动子区域，ft衣壳蛋白的控制区域、3-磷酸甘油酸激酶或其他糖酵解酶的启动子、酸性磷酸酶例如Pho5的启动子、酵母.alpha.接合因子的启动子、杆状病毒系统的多面体启动子，以及已知控制原核和真核细胞以及它们的病毒的基因表达的其他序列，以及它们的各种组合。要理解的是，表达载体的设计可以取决于这些因素，如要转化的宿主细胞的选择，和/或希望表达的蛋白质的类型。此外，还应当考虑载体的拷贝数、控制拷贝数的能力以及载体编码的任何其他蛋白质的表达，例如抗生素标志物。

[0228] 适用于本文描述的方法和组合物的ActRIIA多肽的生产中使用的重组核酸可以通过将克隆的基因或其部分连接到适合于在原核细胞、真核细胞(酵母、禽类、昆虫或哺乳动物)或两者中表达的载体中来产生。用于重组ActRIIA多肽的生产的表达载体包括质粒和其他载体。例如，适合的载体包括质粒类型：pBR322衍生的质粒、pEMBL衍生的质粒、pEX衍生的质粒、pBTac衍生的质粒和pUC-衍生的质粒，用于在原核细胞例如E.coli中表达。

[0229] 某些哺乳动物表达载体含有原核序列来促进载体在细菌中的增殖，以及含有一种或更多种在真核细胞中表达的真核转录单位。pcDNAI/amp、pcDNAI/neo、pRc/CMV、pSV2gpt、pSV2neo、pSV2-dhfr、pTk2、pRSVneo、pMSG、pSVT7、pko-neo和pHyg衍生的载体是适合于转染真核细胞的哺乳动物表达载体的实例。这些载体的一些被来自细菌质粒例如pBR322表达序

列修饰，来促进在原核和真核细胞两者中的复制和药物抗性选择。做为选择，病毒的衍生物，例如，牛乳头状瘤病毒(BPV-1)或Epstein-Barr病毒(pHEBo、pREP-衍生的和p205)可以用于蛋白质在真核细胞中的短暂表达。其他病毒(包括逆转录病毒)表达系统的实例可以在下文基因治疗递送系统的说明中找到。制备质粒和转化宿主生物体所采用的各种方法是本领域公知的。对于原核和真核细胞都适合的其他表达系统，以及一般的重组过程，参见Molecular Cloning A Laboratory Manual,3rd Ed.,ed.by Sambrook,Fritsch and Maniatis(Cold Spring Harbor Laboratory Press,2001)。在某些情况下，可能希望的是通过使用杆状病毒表达系统来表达重组多肽。这样的杆状病毒表达系统的实例包括pVL衍生的载体(例如，pVL1392、pVL1393和pVL941)、pAcUW衍生的载体(例如pAcUW1)和pBlueBac-衍生的载体(例如含有.beta.-gal的pBlueBac III)。

[0230] 载体可以被设计用于在CHO细胞中生产目标ActRIIA多肽，例如，Pcmv-Script载体(Stratagene,La Jolla,Calif.)、pcDNA4载体(Invitrogen,Carlsbad,Calif.)和pCI-neo载体(Promega,Madison,Wis.)。明显的是，目标基因构建体可以用于引起目标ActRIIA多肽在培养物中增殖的细胞中表达，例如，来产生蛋白质，包括融合蛋白或变体蛋白，用于纯化。

[0231] 用包括一种或更多种目标ActRIIA多肽的编码序列(例如，SEQ ID NO:4或5)的重组基因转染的宿主细胞可以用于生产适用于本文描述的方法和组合物的ActRIIA多肽。所述宿主细胞可以是任何原核或真核细胞。例如，本文提供的ActRIIA多肽可以在细菌细胞例如E.coli、昆虫细胞(例如，使用杆状病毒表达系统)、酵母或哺乳动物细胞中表达。其他适合的宿主细胞是本领域技术人员公知的。

[0232] 因而，本文提供的是生产ActRIIA多肽的方法。例如，用编码ActRIIA多肽的表达载体转染的宿主细胞可以在适当的条件下培养来容许发生ActRIIA多肽的表达。从细胞与含有ActRIIA多肽的培养基的混合物中，可以分泌和分离ActRIIA多肽。做为选择，ActRIIA多肽可以细胞质地或在膜部分中保持，收获细胞、裂解并分离蛋白。细胞培养物包括宿主细胞、培养基和其他副产物。用于细胞培养的适合的培养基是本领域公知的。利用本领域已知的用于纯化蛋白质的技术，可以从细胞培养介质、宿主细胞或两者分离目标ActRIIA多肽，包括离子交换层析、凝胶过滤层析、超滤、电泳、使用特异于ActRIIA多肽的特定表位的抗体的免疫亲和纯化、以及使用结合与ActRIIA多肽融合的结构域的试剂的亲和纯化(例如，蛋白A柱可以用于纯化ActRIIA-Fc融合物)。在优选的实施方式中，ActRIIA多肽是含有便于其纯化的结构域的融合蛋白。在一个实施方式中，纯化通过一系列柱层析步骤来实现，包括，例如，三种或更多种以下的，按任何顺序：蛋白A层析、Q琼脂糖层析、苯基琼脂糖层析、空间排阻层析和阳离子交换层析。纯化可以用病毒过滤和缓冲液交换来完成。如本文表明的，ActRIIA-hFc蛋白可以纯化到通过空间排阻层折测定的>98％的纯度，和通过SDS-PAGE测定的>95％的纯度。这一水平的纯度足以在小鼠中对骨骼实现期望的效果，以及在小鼠、大鼠和非人灵长类中实现可接受的安全分布。

[0233] 在另一个实施方式中，编码位于重组ActRIIA多肽的期望部分的N-末端的纯化前导序列例如聚-(His)/肠激酶裂解位点序列的融合基因可以容许表达的融合蛋白通过利用Ni^{2+}金属树脂的亲和层析来纯化。纯化前导序列然后通过用肠激酶处理来除去，以提供纯化的ActRIIA多肽(例如，参见Hochuli et al.,(1987)J.Chromatography 411:177;和Janknecht et al.,PNAS USA 88:8972)。

[0234] 用于制造融合基因的技术是公知的。基本上，根据常规技术进行编码不同多肽序列的各种DNA片段的连接，采用钝末端的或交错末端的末端用于连接，限制酶消化来提供适当的末端，视情况填充粘末端，碱性磷酸酶处理来避免不希望的连接，以及酶连接。在另一个实施方式中，融合基因可以通过常规技术，包括自动的DNA合成仪来合成。做为选择，可以使用锚引物来进行基因片段的PCR扩增，所述锚引物产生两个连续基因片段之间的互补的突出，其随后可以退火来产生嵌合基因序列(参见，例如Current Protocols in Molecular Biology,eds.Ausubel et al.,John Wiley&Sons:1992)。

[0235] ActRIIA-Fc融合蛋白可以从pAID4载体(SV40ori/增强子，CMV启动子)在稳定地转染的CHO-DUKX B1 1细胞中表达，使用SEQ ID NO:9的组织血纤蛋白溶酶原前导序列。Fc部分是人IgG1Fc序列，如SEQ ID NO:7所示。在某些实施方式中，在表达时，平均起来，所含的蛋白质具有每分子的ActRIIA-Fc融合蛋白约1.5到2.5摩尔的唾液酸。

[0236] 在某些实施方式中，ActRIIA-Fc融合物的长血清半衰期可以是在人类对象中25-32天。另外，相比在人293细胞中表达的ActRIIA-hFc融合蛋白所报告的，CHO细胞表达的材料可以具有更高的对激活素B配体的亲和力(del Re et al.,J Biol Chem.2004Dec 17;279(51):53126-35)。另外，不受理论的限制，使用比其他前导序列提供更高生产的TPA前导序列，不同于用天然前导区表达的ActRIIA-Fc，可以提供高纯度的N-末端序列。使用天然前导序列可能产生两种主要种类的ActRIIA-Fc，各自具有不同的N-末端序列。

[0237] 8.5.2 ACTRIIB信号转导的抑制剂

[0238] 如本文使用的，术语“ActRIIB”是指来自任何物种的激活素IIB型受体(ActRIIB)蛋白的家族，以及通过诱变或其他修饰衍生自这些ActRIIB蛋白的变体。本文提及ActRIIB被理解为是提及所述受体的当前鉴定的形式的任一种。ActRIIB家族的成员一般是跨膜的蛋白质，由具有富半胱氨酸区的配体结合细胞外结构域、跨膜结构域、以及具有预测的丝氨酸/苏氨酸激酶活性的细胞质结构域组成。

[0239] 本文描述的组合物和方法中使用的ActRIIB信号转导抑制剂包括，非限制性地，激活素结合可溶ActRIIB多肽；结合激活素(特别是激活素A或B亚基，也称为β_A或β_B)并破坏ActRIIB结合的抗体；结合ActRIIB并破坏激活素结合的抗体；为激活素或ActRIIB结合选择的非抗体蛋白质；以及为激活素或ActRIIB结合选择的随机肽，其可以轭合到Fc结构域。

[0240] 在某些实施方式中，具有激活素或ActRIIB结合活性的两种或更多种不同的蛋白质(或其他部分)，特别是分别阻断I型(例如，可溶的I型激活素受体)和II型(例如，可溶的II型激活素受体)结合位点的激活素结合物可以连接在一起，产生抑制ActRIIB信号转导的双功能或多功能结合分子，因而可以用于本文描述的组合物和方法。在某些实施方式中，抑制ActRIIB的激活素-ActRIIB信号转导轴拮抗剂包括核酸适体、小分子和其他试剂，用于本文描述的组合物和方法中。

[0241] 8.5.2.1 包含ActRIIB多肽的ActRIIB信号转导抑制剂

[0242] 如本文使用的，术语“ActRIIB多肽”是指这些多肽，其包含ActRIIB家族成员的任何天然发生的多肽以及保持了有用活性的其任何变体(包括突变体、片段、融合物和肽模拟物形式)例如，ActRIIB多肽包括一些多肽，其衍生自任何已知的ActRIIB受体的序列、具有至少约80％相同于ActRIIB多肽的序列的序列，任选地至少85％、90％、95％、96％、97％、98％、99％或更高的同一性。例如，ActRIIB多肽可以结合并抑制ActRIIB蛋白质和/或激活

素的功能。ActRIIB多肽的实例包括人ActRIIB前体多肽(SEQ ID NO:16或SEQ ID NO:28)。对于氨基酸序列描述为SEQ ID NO:16或SEQ ID NO:28的ActRIIB前体多肽(即,人ActRIIB前体多肽),ActRIIB前体多肽的信号肽位于氨基酸1到18;细胞外结构域位于氨基酸19到134,潜在的N-连接的糖基化位点位于氨基酸位置42和65。编码SEQ ID NO:16的人ActRIIB前体多肽的核酸序列以SEQ ID NO:19公开(SEQ ID NO:19提供了在相应于氨基酸位置64的密码子处的丙氨酸,但是可以由本领域技术人员使用本领域已知的方法容易地修饰,来提供相应于氨基酸位置64的密码子处的精氨酸作为替代)。序列的描述参见表21。

[0243] 本文描述的所有ActRIIB相关的多肽的氨基酸编号是基于SEQ ID NO:16和SEQ ID NO:28(其仅在位置64表达的氨基酸不同)的氨基酸编号,除非另外指定。例如,如果一ActRIIB多肽被描述为在氨基酸位置79具有取代/突变,这将被理解为该位置79是指SEQ ID NO:16或SEQ ID NO:28中的第79个氨基酸,从其中衍生出所述ActRIIB多肽。同样地,如果一种ActRIIB多肽被描述为在氨基酸位置64具有丙氨酸或精氨酸,这将被理解为位置64是指SEQ ID NO:16或SEQ ID NO:28中的第64个氨基酸,从其中衍生出所述ActRIIB多肽。

[0244] 在某些实施方式中,本文描述的组合物和方法中使用的ActRIIB信号转导的抑制剂包含多肽,所述多肽包含ActRIIB的激活素结合结构域。在某些实施方式中,ActRIIB的激活素结合结构域包含ActRIIB的细胞外结构域,或其部分。在具体的实施方式中,ActRIIB的细胞外结构域或其部分是可溶的。ActRIIB多肽的说明性的修饰形式在美国专利申请公开No.20090005308和20100068215中公开了,其公开内容通过完全引用合并在本文中。

[0245] 在具体的实施方式中,本文描述的组合物和方法中使用的ActRIIB多肽是可溶的ActRIIB多肽。术语“可溶的ActRIIB多肽”一般是指包含ActRIIB蛋白的细胞外结构域的多肽,包括ActRIIB蛋白的任何天然发生的细胞外结构域以及其任何变体(包括突变体、片段和肽模拟物形式)。可溶的ActRIIB多肽可以结合激活素;然而,在结合激活素与GDF8/11方面野生型ActRIIB蛋白不展现显著的选择性。在某些实施方式中,具有不同结合性质的改变形式的ActRIIB可以用于本文提供的方法中。这样的改变的形式公开于,例如,国际专利申请公开No.WO 2006/012627和WO 2010/019261,其公开内容通过完全引用合并在本文中。通过将它们与第二激活素选择性结合试剂偶联,天然的或改变的ActRIIB蛋白可以被给与添加的对激活素的特异性。示范性的可溶的ActRIIB多肽包括人ActRIIB多肽的细胞外结构域(例如,SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43)。

[0246] 具有Hilden et al.(Blood,1994,83(8):2163-70)公开的ActRIIB细胞外序列的Fc融合蛋白,其在相应于ActRIIB前体氨基酸序列即SEQ ID NO:16的氨基酸64的位置具有丙氨酸(本文称为“A64”),已经被证明具有对激活素和GDF-11的相对低的亲和力。相比之下,在ActRIIB前体氨基酸序列的位置64处具有精氨酸(本文称为“R64”)的Fc融合蛋白具有处于低纳摩尔到高皮摩尔范围内的对激活素和GDF-11的亲和力(参见,例如,美国专利申请公开或20100068215,其公开内容完全合并在本文中)。在位置64具有精氨酸的ActRIIB前体氨基酸序列在SEQ ID NO:28中示出。因而,在某些实施方式中,根据本文描述的组合物和方法使用的ActRIIB多肽可以包含(在相应于ActRIIB前体氨基酸序列的氨基酸64的位置上的丙氨酸,即SEQ ID NO:16;或(ii)在ActRIIB前体序列的位置64的精氨酸,即SEQ ID NO:28)。在其他实施方式中,根据本文描述的组合物和方法使用的ActRIIB多肽可以包含在相应于ActRIIB前体氨基酸序列,即SEQ ID NO:16或SEQ ID NO:28的氨基酸64的位置上的不

是丙氨酸或精氨酸的氨基酸。

[0247] 已经显示的是，删除处于ActRIIB的细胞外结构域的C-末端的脯氨酸结降低了受体对激活素的亲和力(参见，例如，Attisano et al.,Cell,1992,68(1):97-108)。相对于包括脯氨酸结区域和完整的近膜结构域、含有SEQ ID NO:28的氨基酸20-134的ActRIIB-Fc融合蛋白“ActRIIB(20-134)-Fc”，含有SEQ ID NO:28的氨基酸20-119(即，SEQ ID NO:32)的ActRIIB-Fc融合蛋白“ActRIIB(20-119)-Fc”具有降低的对GDF-11和激活素的结合。然而，相对于ActRIIB的未截短的细胞外结构域，含有SEQ ID NO:28的氨基酸20-129的ActRIIB-Fc融合蛋白“ActRIIB(20-129)-Fc”保持了相似的但稍微降低的活性，尽管其脯氨酸结区域被破坏。因而，包含在SEQ ID NO:28的氨基酸134、133、132、131、130和129处停止的细胞外结构域的ActRIIB多肽都被预期是有活性的，但是在氨基酸134或133停止的构建体可能具有最大活性。类似地，在残基129-134的任一个上的突变预期不会大幅度改变配体结合亲和力，如SEQ ID NO:28的P129和P130的突变实质上不降低配体结合的事实所指示的。因此，根据本文描述的方法和组合物使用的ActRIIB多肽可以早至SEQ ID NO:28(或SEQ ID NO:16)的氨基酸109(即，最后的半胱氨酸)结束，然而，终止于SEQ ID NO:28(或SEQ ID NO:16)的氨基酸位置109到119或这之间的形式预计不具有降低的配体结合能力。

[0248] SEQ ID NO:16和SEQ ID NO:28的氨基酸29代表ActRIIB前体序列中的最初的半胱氨酸。预计的是，在SEQ ID NO:16或SEQ ID NO:28的N-末端的氨基酸29开始或在这些氨基酸位置之前开始的ActRIIB将保持配体结合活性。在SEQ ID NO:16或SEQ ID NO:28的位置24处丙氨酸到天冬酰胺的突变引入N-连接的糖基化位点而基本上不影响配体结合。这确认了在相应于SEQ ID NO:16或SEQ ID NO:28的氨基酸20-29的信号裂解肽和半胱氨酸交联区之间的突变是良好耐受的。特别地，在SEQ ID NO:16或SEQ ID NO:28的氨基酸位置20、21、22、23和24开始的ActRIIB多肽将保持活性，在SEQ ID NO:16或SEQ ID NO:28的氨基酸位置25、26、27、28和29开始的ActRIIB多肽也预期保持活性。在SEQ ID NO:16或SEQ ID NO:28的氨基酸位置22、23、24或25开始的ActRIIB多肽将具有最大活性。

[0249] 合起来，根据本文描述的方法和组合物使用的ActRIIB前体蛋白(即，SEQ ID NO:16或SEQ ID NO:28)的活性部分(即，ActRIIB多肽)一般将包含SEQ ID NO:16或SEQ ID NO:28的氨基酸29-109，这样的ActRIIB多肽可以，例如，开始于与SEQ ID NO:16或SEQ ID NO:28的氨基酸19-29的任一个相应的残基，结束于与SEQ ID NO:16或SEQ ID NO:28的氨基酸109-134的任一个相应的位置。本文涵盖的ActRIIB多肽的具体实例包括开始于SEQ ID NO:16或SEQ ID NO:28的19-29、20-29或21-29的氨基酸位置、结束于SEQ ID NO:16或SEQ ID NO:28的119-134、119-133或129-134、129-133的氨基酸位置的那些。本文涵盖的ActRIIB多肽的其他具体实例包括开始于SEQ ID NO:16或SEQ ID NO:28的20-24(或21-24，或22-25)的氨基酸位置、结束于SEQ ID NO:16或SEQ ID NO:28的109-134(或109-133)、119-134(或119-133)或129-134(或129-133)的氨基酸位置的那些。落入这些范围的变体ActRIIB多肽也是期待的，特别是与SEQ ID NO:16或SEQ ID NO:28的相应部分具有至少80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％的序列同一性或序列同源性的那些。

[0250] 在某些实施方式中，本文描述的组合物和方法中使用的ActRIIB信号转导的抑制剂包含ActRIIB的细胞外结构域的截短的形式。所述截短可以是在ActRIIB多肽的羧基末端

和/或氨基末端。在某些实施方式中，相对于成熟的ActRIIB多肽细胞外结构域，所述截短可以是1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25个氨基酸长度。在某些实施方式中，所述截短可以是1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25个成熟ActRIIB多肽细胞外结构域的N-末端氨基酸。在某些实施方式中，所述截短可以是1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25个成熟ActRIIB多肽细胞外结构域的C-末端氨基酸。例如，ActRIIB的截短形式包括具有氨基酸20-119；20-128；20-129；20-130；20-131；20-132；20-133；20-134；20-131；21-131；22-131；23-131；24-131；和25-131的多肽，其中所述氨基酸位置参考SEQ ID NO:16或SEQ ID NO:28中的氨基酸位置。

[0251] ActRIIB的其他示范性的截短形式包括(i)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸21-29的任一个的氨基酸(任选地开始于SEQ ID NO:16或SEQ ID NO:28的21-25)以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸109-134的任一个的多肽；(ii)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸20-29的任一个(任选地开始于SEQ ID NO:16或SEQ ID NO:28的20-25)以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸109-133的任一个的多肽；(iii)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸20-24的任一个(任选地开始于SEQ ID NO:16或SEQ ID NO:28的20-25)以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸109-133的任一个的多肽；(iv)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸21-24的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸109-134的任一个的多肽；(v)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸20-24的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸118-133的任一个的多肽；(vi)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸21-24的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸118-134的任一个的多肽；(vii)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸20-24的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸128-133的任一个的多肽；(viii)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸20-24的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸128-133的任一个的多肽；(ix)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸21-29的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸118-134的任一个的多肽；(x)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸20-29的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸118-133的任一个的多肽；(xi)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸21-29的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸128-134的任一个的多肽；和(xii)开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸20-29的任一个以及结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸128-133的任一个的多肽。在具体的实施方式中，ActRIIB多肽包含，基本上由以下组成，或由以下组成，开始于SEQ ID NO:16或SEQ ID NO:28的氨基酸位置25、并结束于SEQ ID NO:16或SEQ ID NO:28的氨基酸位置131的氨基酸序列。在另一个具体的实施方式中，ActRIIB由以下组成或基本上由以下组成，SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42或43的氨基酸序列。

[0252] 本文描述的组合物和方法中使用的ActRIIB多肽的任一个可以作为同二聚体生产。本文描述的组合物和方法中使用的ActRIIB多肽的任一个可以被配制为具有异源部分的融合蛋白，其包含来自IgG重链的恒定区，例如Fc结构域。本文描述的组合物和方法中使用的ActRIIB多肽的任一个可以包含处在相应于SEQ ID NO:16或SEQ ID NO:28的位置79的

位置上的酸性氨基酸，任选地与相对于SEQ ID NO:16或SEQ ID NO:28的一个或更多个其他的氨基酸取代、删除或插入组合。

[0253] 在具体的实施方式中，本文描述的组合物和方法中使用的ActRIIB信号转导的抑制剂包含具有一个或更多个氨基酸取代/突变的ActRIIB的细胞外结构域。这样的氨基酸取代/突变可以是，例如，SEQ ID NO:16或SEQ ID NO:28的氨基酸位置79处的亮氨酸交换为酸性氨基酸，例如，天冬氨酸或谷氨酸。例如，SEQ ID NO:16或SEQ ID NO:28的位置L79可以在ActRIIB细胞外结构域多肽中改变，来赋予改变的激活素-肌肉生长抑制素(GDF-11)结合性质。相比激活素结合，L79A和L79P突变更大程度地降低GDF-11结合。L79E和L79D突变保持了GDF-11结合，而展现了大大地降低的激活素结合。

[0254] 在某些实施方式中，本文描述的组合物和方法中使用的ActRIIB信号转导的抑制剂包含截短形式的ActRIIB细胞外结构域，其还带有氨基酸取代，例如，SEQ ID NO:16或SEQ ID NO:28的氨基酸位置79处的亮氨酸改变为酸性氨基酸，例如，天冬氨酸或谷氨酸。在具体的实施方式中，本文描述的组合物和方法中使用的还带有氨基酸取代的ActRIIB多肽的细胞外结构域的截短形式是SEQ ID NO:23。截短的和/或带有一个或更多个氨基酸取代的ActRIIB的形式可以如以上讨论的连接到抗体的Fc结构域。

[0255] 例如，通过筛选重组地产自编码ActRIIB多肽的核酸的相应片段的多肽，可以获得ActRIIB多肽的功能活性片段。此外，片段可以使用本领域已知的技术例如，常规的Merrifield固相f-Moc或t-Boc化学作用来化学地合成。本领域技术人员将认识到的是，其他技术，包括例如用于肽合成的各种树脂和液相系统可以用于合成本文描述的肽基片段。所述片段可以被产生(重组地或通过化学合成)和测试，来鉴定可以用作ActRIIB蛋白或激活素介导的信号转导的拮抗剂(抑制剂)的那些肽片段。

[0256] 此外，例如，通过筛选重组地产自编码ActRIIB多肽的相应诱变核酸的修饰多肽的文库，可以获得ActRIIB多肽的功能活性的变体。所述变体可以被产生和测试，来鉴定可以用作ActRIIB蛋白的或激活素介导的信号转导的拮抗剂(抑制剂)的那些。在某些实施方式中，ActRIIB多肽的功能变体包括至少75％相同于选自SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的氨基酸序列的氨基酸序列。在某些实施方式中，所述功能变体具有至少80％、85％、90％、95％、96％、97％、98％或99％相同于选自SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的氨基酸序列的氨基酸序列。

[0257] 例如，通过为了诸如增强效力或稳定性(例如，体外货架寿命或对体内蛋白水解降解的抗性)的目的修饰ActRIIB多肽的结构，可以产生功能变体。当被选择以保持激活素结合时，这种修饰的ActRIIB多肽可以被认为是天然发生的ActRIIB多肽的功能等效物。例如，通过氨基酸取代、删除或添加，也可以产生修饰的ActRIIB多肽。例如，合理的是预计，亮氨酸用异亮氨酸或缬氨酸、天冬氨酸用谷氨酸、苏氨酸用丝氨酸的独立替换，或氨基酸用结构相关氨基酸的相似性替换(例如，保守突变)将不会对产生的分子的生物学活性有重大的影响。保守性替换是在其侧链相关的氨基酸家族内发生的替换。通过评估变体ActRIIB多肽在细胞中以类似于野生型ActRIIB多肽的方式产生反应的能力，ActRIIB多肽的氨基酸序列改变是否产生功能性同源物可以容易地测定。

[0258] ActRIIB多肽突变体，特别是ActRIIB多肽的组合突变体的集合，以及截短突变体；组合突变体的库，对于鉴定可用于本文描述的方法和组合物的功能变体序列是特别有用

的。筛选这种组合文库的目的可以是产生，例如，可以用作激动剂或拮抗剂的ActRIIB多肽变体，或作为选择，其一起具有新的活性。

[0259] 已经证明的是，由SEQ ID NO:16或SEQ ID NO:28的残基Y31、N33、N35、L38到T41、E47、E50、Q53到K55、L57、H58、Y60、S62、K74、W78到N83、Y85、R87、A92和E94到F101定义了ActRIIB的配体结合口袋。在这些位置上，预计的是保守性突变将是耐受的，而K74A突变是良好耐受的，R40A、K55A、F82A和位置L70的突变也是。在非洲蟾蜍(Xenopus)中R40是K，表明这个位置的碱性氨基酸是耐受的。在牛ActRIIB中Q53是R，在非洲蟾蜍ActRIIB中是K，因而包括R、K、Q、N和H的氨基酸在这个位置将是耐受的。因而，用于本文描述的方法和组合物的ActRIIB多肽的通式是，包含SEQ ID NO:16或SEQ ID NO:28的氨基酸29-109，但任选地开始于SEQ ID NO:16或SEQ ID NO:28的20-24或22-25的氨基酸位置，以及结束于SEQ ID NO:16或SEQ ID NO:28的129-134的氨基酸位置，在配体结合口袋中包含不超过1、2、5或15个保守氨基酸改变，以及在配体结合口袋中SEQ ID NO:16或SEQ ID NO:28的氨基酸位置40、53、55、74、79和/或82的零个、一个或更多个非保守改变。这样的ActRIIB多肽可以保持与SEQ ID NO:16或SEQ ID NO:28的氨基酸29-109的序列大于80％、90％、95％或99％的序列同一性或序列同源性。结合口袋之外的位点，在此变异性是特别好地耐受的，包括ActRIIB的细胞外结构域的氨基和羧基末端，以及位置42-46和65-73。SEQ ID NO:16或SEQ ID NO:28的位置65处天冬酰胺到丙氨酸的改变(N65A)实际上改善了A64背景下的配体结合，因而预计在R64背景下对配体结合没有不利影响。这种改变可能除去了A64背景下在N65处的糖基化，因而表明这个区域的显著改变可能是耐受的。虽然R64A改变是不良耐受的，R64K是良好耐受的，因而另一种碱性残基，例如，H可以在位置64处耐受。

[0260] 作为在配体结合结构域中有突变的ActRIIB多肽的具体实例，ActRIIB的配体结合结构域的带正电氨基酸残基Asp(D80)可以突变为不同的氨基酸残基，从而变体ActRIIB多肽优先地结合GDF8而不是激活素。在具体的实施方式中，D80残基被改变为选自以下构成的组的氨基酸残基：不带电的氨基酸残基、负电氨基酸残基和疏水性氨基酸残基。作为进一步的具体实例，疏水性残基L79可以改变为酸性氨基酸天冬氨酸或谷氨酸，来大大降低激活素结合而保持GDF11结合。本领域技术人员将认识到，大多数所描述的突变体、变体或修饰体可以在核酸水平制备，或在某些情况下，通过翻译修饰或化学合成制备。这样的技术是本领域公知的。

[0261] 在具体的实施方式中，本文描述的组合物和方法中使用的ActRIIB信号转导的抑制剂包含靶合物/融合蛋白，其包含与抗体的Fc部分连接的ActRIIB受体的细胞外结构域(例如，激活素结合结构域)。这样的靶合物/融合蛋白可以包含本文公开的任何ActRIIB多肽(例如，SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42或43的任一个)、任何本领域已知的ActRIIB多肽或使用本领域已知的和/或本文提供的方法产生的任何ActRIIB多肽。

[0262] 在某些实施方式中，细胞外结构域通过接头，例如肽接头连接到抗体的Fc部分。示范性的接头包括短多肽序列，例如2-10、2-5、2-4、2-3个氨基酸残基(例如，甘氨酸残基)，例如，Gly-Gly-Gly接头。在具体的实施方式中，所述接头包含氨基酸序列Gly-Gly-Gly(GGG)。在另一个具体的实施方式中，所述接头包含氨基酸序列Thr-Gly-Gly-Gly(TGGG)。任选地，所述Fc结构域在残基例如Asp-265、赖氨酸322和Asn-434处具有一个或更多个突变。在某些

情况下，具有一个或更多个这些突变(例如，Asp-265突变)的突变Fc结构域具有相对于野生型Fc结构域降低的结合Fcγ受体的能力。在其它情况下，具有一个或更多个这些突变(例如，Asn-434突变)的突变Fc结构域相对于野生型Fc结构域具有提高的结合MHC I类相关Fc受体(FcRN)的能力。包含与Fc结构域融合的ActRIIB的可溶细胞外结构域的示范性的融合蛋白在SEQ ID NO:20、21、24、25、34、35、38、39、40、41、44、46和47中列出。

[0263] 在具体的实施方式中，本文描述的组合物和方法中使用的ActRIIB信号转导抑制剂包含与抗体的Fc部分连接的ActRIIB的细胞外结构域，或其部分，其中所述ActRIIB信号转导抑制剂包含至少75％相同于选自SEQ ID NO:20、21、24、25、34、35、38、39、40、41、44、46和47的氨基酸序列的氨基酸序列。在另一个具体的实施方式中，本文描述的组合物和方法中使用的ActRIIB信号转导抑制剂包含与抗体的Fc部分连接的ActRIIB的细胞外结构域，或其部分，其中所述ActRIIB信号转导抑制剂包含至少80％、85％、90％、95％、96％、97％、98％或99％相同于选自SEQ ID NO:20、21、24、25、34、35、38、39、40、41、44、46和47的氨基酸序列的氨基酸序列。

[0264] 在具体的实施方式中，用于本文描述的组合物和方法的ActRIIB信号转导抑制剂是人ActRIIB受体的细胞外结构域与IgG1的Fc部分之间的融合蛋白。在另一个具体的实施方式中，用于本文描述的组合物和方法的ActRIIB信号转导抑制剂是人ActRIIB受体的截短的细胞外结构域与IgG1的Fc部分之间的融合蛋白。在另一个具体的实施方式中，用于本文描述的组合物和方法的ActRIIB信号转导抑制剂是人ActRIIB受体的截短的细胞外结构域与IgG1的Fc部分之间的融合蛋白，其中所述人ActRIIB受体的截短的细胞外结构域具有相应于SEQ ID NO:16或SEQ ID NO:28的氨基酸79的氨基酸位置处的氨基酸取代。在一个实施方式中，在相应于SEQ ID NO:16或SEQ ID NO:28的氨基酸79的氨基酸位置处的氨基酸取代是亮氨酸到天冬氨酸的取代(即，L79D突变)。

[0265] 在具体的实施方式中，用于本文描述的组合物和方法的ActRIIB信号转导抑制剂是SEQ ID NO:24或25，其代表人ActRIIB受体的细胞外结构域与IgG1的Fc部分之间的融合蛋白，其中所述ActRIIB细胞外结构域包含带有L79D突变的SEQ ID NO:28的氨基酸25-131。编码SEQ ID NO:24的ActRIIB-Fc融合蛋白的核酸序列在SEQ ID NO:45中示出。

[0266] 在另一个具体的实施方式中，用于本文描述的组合物和方法的ActRIIB信号转导抑制剂是SEQ ID NO:34或35，其代表人ActRIIB受体的细胞外结构域与IgG1的Fc部分之间的融合蛋白，其中所述ActRIIB细胞外结构域包含带有L79D突变的SEQ ID NO:16的氨基酸25-131。

[0267] 天冬酰胺连接的糖基化识别位点一般包含三肽序列，天冬酰胺-X-苏氨酸(或天冬酰胺-X-丝氨酸)(其中“X”是任意氨基酸)，其被适当的细胞糖基化酶特异性地识别。也可以通过向野生型ActRIIB多肽的序列中添加或取代一个或多个丝氨酸或苏氨酸残基(用于O—连接的糖基化位点)来进行改变。在糖基化识别位点的第一或第三个氨基酸位置的一个或两个上的各种氨基酸取代或删除(和/或在第二位置上的氨基酸删除)引起所述修饰的三肽序列处的非糖基化。提高ActRIIB多肽上碳水化合物部分的数量的另一种方式是通过糖苷对ActRIIB多肽的化学或酶学偶联。取决于使用的偶联方式，糖可以附着于(a)精氨酸和组氨酸；(b)游离羧基基团；(c)游离巯基基团，例如半胱氨酸的；(d)游离羧基基团，例如，丝氨酸、苏氨酸或羟脯氨酸的；(e)芳香族残基，例如苯丙氨酸、酪氨酸或色氨酸的；或(f)谷氨酰

胺的酰胺基团。这些方法在1987年9月11日公开的WO 87/05330中，以及在Aplin and Wriston(1981)CRC Crit.Rev.Biochem.,pp.259-306中描述了，通过引用合并在本文中。除去ActRIIB多肽上存在的一个或更多个碳水化合物部分可以化学地或酶学地实现。化学的脱糖基化可能涉及例如将ActRIIB多肽暴露于化合物三氟甲磺酸或相当的化合物。这种处理引起除了连接的糖(N-乙酰葡萄糖胺或N-乙酰半乳糖胺)之外大部分或所有的糖的裂解，而保留氨基酸序列完整。化学的脱糖基化由Hakimuddin et al.(1987)Arch.Biochem.Biophys.259:52和Edge et al.(1981)Anal.Biochem.118:131进一步描述了。ActRIIB多肽上碳水化合物部分的酶裂解可以通过使用多种内切和外切糖苷酶来实现，如Thotakura et al.(1987)Meth.Enzymol.138:350所描述的。ActRIIB多肽的序列可以被调整，视情况，取决于使用的表达系统的类型，因为哺乳动物、酵母、昆虫和植物细胞可能都引入不同的糖基化模式，其可能受到肽的氨基酸序列的影响。一般地，用于人类的ActRIIB蛋白将在提供适当的糖基化的哺乳动物细胞系中表达，例如，HEK293或CHO细胞系，而其他表达系统，例如其他哺乳动物表达细胞系、具有工程化的糖基化酶的酵母细胞系以及昆虫细胞预计也是有用的。

[0268] 在具体的实施方式中，相对于ActRIIB(R64)-Fc形式，包含了提高ActRIIB-Fc融合蛋白的血清半衰期的进一步的N-连接的糖基化位点(N-X-S/T)的突变的ActRIIB多肽可以用于本文描述的方法和组合物中。在具体的实施方式中，在SEQ ID NO:16或SEQ ID NO:28的位置24处引入天冬酰胺(A24N)产生了赋予更长半衰期的NXT序列。其他NX(T/S)序列可以在42-44(NQS)和65-67(NSS)中找到，而后者可能不不能用位置64的R有效地糖基化(即，在R64多肽中)。N-X-S/T序列一般可以引入到ActRIIB的配体结合口袋之外的位置，其在上文中详述了。对于引入非内源的N-X-S/T序列特别适合的位点包括SEQ ID NO:16或SEQ ID NO:28的氨基酸20-29、20-24、22-25、109-134、120-134或129-134。N-X-S/T序列还可以导入到ActRIIB序列与Fc或其他融合部件之间的接头中。因而，产生N-连接的糖基化位点的可取的改变是：A24N、R64N、S67N(可能与N65A改变组合)、E106N、R112N、G120N、E123N、P129N、A132N、R112S和R112T(所有氨基酸位置相应于它们在SEQ ID NO:16或SEQ ID NO:28中的位置)。预计诶糖基化的任何S可以改变为T，而不产生免疫原性位点，因为由糖基化提供了保护。同样地，预计被糖基化的任何T可以改变为S。因而，改变S67T和S44T被涵盖在此。同样地，在A24N变体中，可以使用S26T改变。因而，ActRIIB多肽可以包括一个或更多个额外的、非内源的N-连接的糖基化共有序列。

[0269] 多种筛选分析可以用于评估ActRIIB多肽变体。例如，可以筛选ActRIIB多肽变体结合ActRIIB配体，防止ActRIIB配体与ActRIIB多肽结合，或干扰由ActRIIB配体引起的信号转导的能力。ActRIIB多肽或其变体活性也可以在基于细胞的或体内的分析中测试。

[0270] 可以产生组合衍生的变体，其相对于天然发生的ActRIIB多肽具有选择的或一般提高的效价。同样，诱变可导致细胞内半衰期与相应的野生型ActRIIB多肽显著不同的变体。例如，改变的蛋白可以变得对引起天然ActRIIB多肽的破坏或灭活的蛋白水解降解或其他细胞代谢过程更稳定或更不稳定。这样的变体和编码它们的基因可以被利用通过调节ActRIIB多肽的半衰期来改变ActRIIB多肽水平。例如，短的半衰期可以产生更短暂的生物学效果，可以容许对象体内重组ActRIIB多肽水平的紧密的控制。在Fc融合蛋白中，突变可以在接头(如果有的话)和/或Fc部分进行，来改变蛋白质的半衰期。

[0271] 通过编码多肽文库的简并基因文库的方式可以产生组合文库，所述多肽文库各自包括可能的ActRIIB多肽序列的至少一部分。例如，合成的寡核苷酸的混合物可以酶学连接到基因序列中，从而可能的ActRIIB多肽核苷酸序列的简并集合可以作为单独的多肽表达，或作为选择，作为更大的融合蛋白的集合(例如，用于噬菌体展示)。

[0272] 有许多方法可以从简并寡核苷酸序列中产生可能的同源物的文库。可以在自动DNA合成仪中进行简并基因序列的化学合成，合成的基因然后可以连接到适合的载体中用于表达。简并寡核苷酸的合成是本领域公知的(参见，例如，Narang,S A(1983)Tetrahedron 39:3;Itakura et al.,(1981)Recombinant DNA,Proc.3rd Cleveland Sympos.Macromolecules,ed.AG Walton,Amsterdam:Elsevier pp 273-289;Itakura et al.,(1984)Annu.Rev.Biochem.53:323;Itakura et al.,(1984)Science 198:1056;Ike et al.,(1983)Nucleic Acid Res.11:477)。这样的技术已经应用于其他蛋白质的定向进化(参见，例如，Scott et al.,(1990)Science 249:386-390;Roberts et al.,(1992)PNAS USA 89:2429-2433;Devlin et al.,(1990)Science 249:404-406;Cwirla et al.,(1990)PNAS USA 87:6378-6382;以及美国专利Nos.5,223,409、5,198,346和5,096,815)。

[0273] 做为选择，可以利用其他形式的诱变来产生组合文库。例如，ActRIIB多肽变体可以通过筛选来从文库中产生和分离，利用，例如，丙氨酸扫描诱变等(Ruf et al.,(1994)Biochemistry 33:1565-1572;Wang et al.,(1994)J.Biol.Chem.269:3095-3099;Balint et al.,(1993)Gene 137:109-118;Grodberg et al.,(1993)Eur.J.Biochem.218:597-601;Nagashima et al.,(1993)J.Biol.Chem.268:2888-2892;Lowman et al.,(1991)Biochemistry 30:10832-10838;和Cunningham et al.,(1989)Science 244:1081-1085)，通过接头扫描诱变(Gustin et al.,(1993)Virology 193:653-660;Brown et al.,(1992)Mol.Cell Biol.12:2644-2652;McKnight et al.,(1982)Science 232:316)；通过饱和诱变(Meyers et al.,(1986)Science 232:613)；通过PCR诱变(Leung et al.,(1989)Method Cell Mol Biol 1:11-19)；或通过随机诱变，包括化学诱变等(Miller et al.,(1992)A Short Course in Bacterial Genetics,CSHL Press,Cold Spring Harbor,N.Y.;和Greener et al.,(1994)Strategies in Mol Biol 7:32-34)。接头扫描诱变，特别是在组合场景中，是鉴定ActRIIB多肽的截短形式(生物活性)的吸引人的方法。

[0274] 大量的技术是本领域已知的，用于筛选由点突变或截短产生的组合库的基因产物，就此而言，用于筛选cDNA库中具有某些性质的基因产物。这些技术一般将适合于通过ActRIIB多肽的组合诱变产生的基因库的快速筛选。用于筛选大的基因文库的最广泛使用的技术一般包括将基因文库克隆到可复制的表达载体中，用产生的载体库转化合适的细胞，并在一定条件下表达组合的基因，在所述条件中期望的活性的检测便于相对容易地分离编码被检出产物的基因的载体。优选的分析包括激活素结合分析和激活素介导的细胞信号转导分析。

[0275] 在某些实施方式中，本文描述的方法和组合物的抑制剂中使用的ActRIIB多肽可以进一步包含翻译后修饰，除了天然存在于所述ActRIIB多肽中的之外。这种的修饰可以包括但不限于，乙酰化、羧基化、糖基化、磷酸化、脂质化和酰化。结果，修饰的ActRIIB多肽可以含有非氨基酸元件，例如，聚乙二醇、脂质、聚糖或单体和磷酸盐。这种非氨基酸元件对ActRIIB多肽的功能的影响可以通过熟练技术人员已知的任何方法来测试。当通过裂解

ActRIIB多肽的新生形式来在细胞中产生ActRIIB多肽时，翻译后的加工对于蛋白质的正确折叠和/或功能可能也是重要的。不同的细胞(例如CHO、HeLa、MDCK、293、W138、NIH-3T3或HEK293)具有这种翻译后活性的特定细胞机制和特征性的机制，可以选择来确保ActRIIB多肽的正确修饰和加工。

[0276] 在某些方面，ActRIIB多肽的功能变体或修饰形式包括具有至少一部分ActRIIB多肽和一个或更多个融合结构域的融合蛋白。这样的融合结构域的公知的实例包括但不限于，多组氨酸、Glu-Glu、谷胱甘肽S转移酶(GST)、硫氧还蛋白、蛋白A、蛋白G、免疫球蛋白重链恒定区(Fc)、麦芽糖结合蛋白(MBP)或人血清白蛋白。可以选择融合结构域以赋予期望的性质。例如，某些融合结构域对于通过亲和层析分离融合蛋白是特别有用的。对于亲和纯化来说，使用亲和层析的相关基质，例如，谷胱甘肽-、淀粉酶-和镍-或钴-轭合的树脂。许多这样的基质是以“试剂盒”形式可获得的，例如，对于(HIS6)融合配体有用的Pharmacia GST纯化系统，以及QIAexpressTM系统(Qiagen)。作为另一个实例，可以选择融合结构域以促进ActRIIB多肽的检测。这样的检测结构域的实例包括各种荧光蛋白(例如，GFP)，以及“表位标签”，其通常是有特异性抗体的短肽序列。特异性单克隆抗体容易获得的公知的表位标签包括FLAG、流感病毒血凝素(HA)和c-myc标签。在某些情况下，融合结构域具有蛋白酶裂解位点，例如，因子Xa或凝血酶的，其容许相关蛋白酶部分地消化所述融合蛋白，从而由此释放重组蛋白。释放的蛋白质然后可以通过随后的层析分离从融合结构域中分离。在某些优选的实施方式中，ActRIIB多肽与使所述ActRIIB多肽在体内稳定化的结构域融合(“稳定物”结构域)。“稳定”是指提高血清半衰期的任何事，不论是由于降低的破坏、降低的肾脏清除、还是其他的药物动力学效应。已知与免疫球蛋白的Fc部分融合为大范围的蛋白质赋予期望的药物动力学性质。同样地，与人血清白蛋白融合可以赋予期望的性质。可以选择的其他类型的融合结构域包括多聚化(例如，二聚化、四聚化)结构域和功能结构域(赋予其他生物学功能的，例如，进一步刺激骨骼生长或肌肉生长，如所希望的)。

[0277] 要理解的是融合蛋白的不同元件可以以与期望的功能一致的任何方式布置。例如，ActRIIB多肽可以置于异源结构域的C-末端，或做为选择，异源结构域可以置于ActRIIB多肽的C-末端。ActRIIB多肽结构域和异源结构域不必在融合蛋白中相邻，其他的结构域或氨基酸序列可以被包括在任一结构域的C-末端或N-末端，或两个结构域之间。

[0278] 在某些实施方式中，本文描述的方法和组合物的抑制剂中使用的ActRIIB多肽可以含有能够稳定化所述ActRIIB多肽的一个或更多个修饰。例如，这样的修饰可以增强ActRIIB多肽的体外半衰期，增强ActRIIB多肽的循环半衰期，或降低ActRIIB多肽的蛋白水解酶降解。这样的稳定化修饰可以包括，但不限于，融合蛋白(包括，例如，包含ActRIIB多肽和稳定化结构域的融合蛋白)、糖基化位点的修饰(包括，例如，向ActRIIB多肽添加糖基化位点)，以及碳水化合物部分的修饰(包括，例如，从ActRIIB多肽除去碳水化合物部分)。对于融合蛋白来说，ActRIIB多肽融合到稳定结构域，例如IgG分子(例如，Fc结构域)。如本文使用的，术语“稳定物结构域”不仅是指融合蛋白情况下的融合结构域(例如，Fc)，还包括非蛋白性的修饰，例如，碳水化合物部分，或非蛋白性的聚合物，例如，聚乙二醇。

[0279] 在某些实施方式中，本文描述的方法和组合物使用分离的或纯化的ActRIIB多肽，即，分离自或基本上没有其他蛋白质的ActRIIB多肽可以用于本文描述的方法和组合物。一般将通过从重组核酸表达来产生ActRIIB多肽。

[0280] 在某些方面，本文描述的方法和组合物中使用的ActRIIB多肽由分离的和/或重组的核酸编码，包括本文公开的片段、功能变体和融合蛋白。例如，SEQ ID NO:19编码天然发生的人ActRIIB前体多肽。目标核酸可以是单链的或是双链的。这样的核酸可以是DNA或RNA分子。这些核酸可以用于，例如，制造ActRIIB多肽的方法，或作为直接的治疗试剂(例如，在基因治疗方法中)。

[0281] 在某些方面，可以用生产适用于本文描述的方法和组合物的ActRIIB多肽的核酸进一步被理解为包括作为SEQ ID NO:19的变体的核酸，以及编码可溶ActRIIB多肽的那些核酸序列的变体(例如，编码SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的核酸)。变体核苷酸序列包括差异在于一个或更多个核苷酸取代、添加或删除的序列，例如，等位变体。

[0282] 在某些实施方式中，可以用于生产适用于本文描述的方法和组合物的ActRIIB多肽的分离的或重组的核酸序列是至少80％、85％、90％、95％、97％、98％、99％或100％相同于SEQ ID NO:19或编码可溶ActRIIB多肽的那些核酸序列(例如，编码SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的核酸)。本领域普通技术人员将理解，与SEQ ID NO:19或编码可溶ActRIIB多肽的那些核酸序列(例如，编码SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的核酸)互补的核酸序列，以及SEQ ID NO:19或编码可溶ActRIIB多肽的那些核酸序列(例如，编码SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的核酸)的变体可以用于本文描述的方法和组合物。在进一步的实施方式中，所述核酸序列可以是分离的，重组和/或融合到异源核苷酸序列的，或是在DNA文库中。

[0283] 在其他实施方式中，可以用于生产适用于本文描述的方法和组合物的ActRIIB多肽的核酸包括在高度严格条件与SEQ ID NO:19中指定的核苷酸序列或编码可溶ActRIIB多肽的那些核酸序列(例如，编码SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的核酸)、SEQ ID NO:19的互补序列或编码可溶ActRIIB多肽的那些核酸序列(例如，编码SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的核酸)或其片段杂交的核苷酸序列。本领域普通技术人员将理解，促进DNA杂交的合适的严格条件可以是变化的。例如，人们可以在6.0倍氯化钠/柠檬酸钠(SSC)、大约45℃下杂交，最后2.0倍SSC在50℃下洗涤。例如，洗涤步骤中的盐浓度可以选自50℃下的约2.0倍SSC的低严格度，到50℃下约0.2倍SSC的高严格度。此外，洗涤步骤中的温度可以从室温约22℃下的低严格条件，提高到大约65℃的高严格条件。温度和盐度可以都变化，或者温度或盐浓度保持不变而另一个变量发生改变。在一个实施方式中，在6倍SSC室温下的低严格条件下杂交、随后在室温下2倍SSC洗涤的核酸可以用于本文描述的方法和组合物。

[0284] 由于遗传密码方面的简并性而不同于SEQ ID NO:19中所列的核酸或编码可溶ActRIIB多肽的那些核酸序列(例如，编码SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的核酸)的分离的核酸也可以用于生产适用于本文描述的方法和组合物的ActRIIB多肽。例如，许多氨基酸由超过一种三联体指定。指定相同氨基酸的密码子，或同义密码子(例如，CAU和CAC对于组氨酸是同义的)可以产生“沉默”突变，其不影响蛋白质的氨基酸序列。然而，预计的是，引起目标蛋白质的氨基酸序列改变的DNA序列多态性将存在于哺乳动物细胞。本领域技术人员将理解，编码特定蛋白质的核酸的一个或更多个核苷酸(达到核苷酸的约3-5％)中的这些变异由于天然的等位变异可以存在于给定物种的个体中。任

何和所有的这类核苷酸变异和产生的氨基酸多态性可以用于本文描述的方法和组合物。

[0285] 在某些实施方式中，可以用于生产适用于本文描述的方法和组合物的ActRIIB多肽的重组核酸可以可操作连接到表达构建体中的一个或更多个调节核苷酸序列。调节核苷酸序列一般将适合于用来表达的宿主细胞。许多类型的适当的表达载体和适合的调节序列是本领域已知的，用于多种宿主细胞。一般地，所述一个或更多个调节核苷酸序列可以包括，但不限于，启动子序列、前导区或信号序列、核糖体结合位点、转录起始和终止序列、翻译起始和终止序列，以及增强子或激活物序列。本领域已知的组成型或可诱导启动子可以用于本文描述的方法和组合物。启动子可以是天然发生的启动子，或组合了超过一种启动子的元件的杂合启动子。表达构建体可以存在于细胞中或游离体例如质粒上，或者表达构建体可以插入染色体中。在优选的实施方式中，表达载体含有选择性标记基因以容许选择转化的宿主细胞。选择性标记基因是本领域公知的，将随使用的宿主细胞而变化。

[0286] 在某些方面，可用于生产适用于本文描述的方法和组合物的ActRIIB多肽的核酸可以在表达载体中提供，所述表达载体包含编码ActRIIB多肽的核苷酸序列并与至少一个调节序列可操作连接。调节序列是公知的，被选择来指导ActRIIB多肽的表达。因而，术语调节序列包括启动子、增强子和其他表达控制元件。示范性的调节序列在Goeddel;Gene Expression Technology:Methods in Enzymology,Academic Press,San Diego,Calif.(1990)中描述。例如，控制与之可操作地连接的DNA序列的表达的各种表达控制序列的任一种，可以用于这些载体来表达编码ActRIIB多肽的DNA序列。这类有用的表达控制序列包括，例如，SV40的早期和晚期启动子、tet启动子、腺病毒或巨细胞病毒即时早期启动子、RSV启动子、lac系统、trp系统、TAC或TRC系统、其表达由T7RNA聚合酶指导的T7启动，噬菌体λ的主要操纵子和启动子区域，ft衣壳蛋白的控制区域、3-磷酸甘油酸激酶或其他糖酵解酶的启动子、酸性磷酸酶例如Pho5的启动子、酵母.alpha.接合因子的启动子、杆状病毒系统的多面体启动子，以及已知控制原核和真核细胞以及它们的病毒的基因表达的其他序列，以及它们的各种组合。要理解的是，表达载体的设计可以取决于这些因素，如要转化的宿主细胞的选择，和/或希望表达的蛋白质的类型。此外，还应当考虑载体的拷贝数、控制拷贝数的能力以及载体编码的任何其他蛋白质的表达，例如抗生素标志物。

[0287] 重组核酸可以通过将克隆的基因或其部分连接到适合于在原核细胞、真核细胞(酵母、禽类、昆虫或哺乳动物)或两者中表达的载体中来产生。用于重组ActRIIB多肽的生产的表达载体包括质粒和其他载体。例如，适合的载体包括质粒类型:pBR322衍生的质粒、pEMBL衍生的质粒、pEX衍生的质粒、pBTac衍生的质粒和pUC-衍生的质粒，用于在原核细胞例如E.coli中表达。

[0288] 某些哺乳动物表达载体含有原核序列来促进载体在细菌中的增殖，以及含有一种或更多种在真核细胞中表达的真核转录单位。pcDNAI/amp、pcDNAI/neo、pRc/CMV、pSV2gpt、pSV2neo、pSV2-dhfr、pTk2、pRSVneo、pMSG、pSVT7、pko-neo和pHyg衍生的载体是适合于转染真核细胞的哺乳动物表达载体的实例。这些载体的一些被来自细菌质粒例如pBR322表达序列修饰，来促进在原核和真核细胞两者中的复制和药物抗性。做为选择，病毒的衍生物，例如，牛乳头状瘤病毒(BPV-1)或Epstein-Barr病毒(pHEBo、pREP-衍生的和p205)可以用于蛋白质在真核细胞中的短暂表达。其他病毒(包括逆转录病毒)表达系统的实例可以在下文基因治疗递送系统的说明中找到。制备质粒和转化宿主生物体所采用的各种方法是本领域公

知的。对于原核和真核细胞都适合的其他表达系统，以及一般的重组过程，参见Molecular Cloning A Laboratory Manual,3rd Ed.,ed.by Sambrook,Fritsch and Maniatis(Cold Spring Harbor Laboratory Press,2001)。在某些情况下，可能希望的是通过使用杆状病毒表达系统来表达重组多肽。这样的杆状病毒表达系统的实例包括pVL衍生的载体(例如，pVL1392、pVL1393和pVL941)、pAcUW衍生的载体(例如pAcUW1)和pBlueBac-衍生的载体(例如含有.beta.-gal的pBlueBac III)。

[0289] 在一个实施方式中，载体可以被设计用于在CHO细胞中生产本文描述的方法和组合物中使用的ActRIIB多肽，例如Pcmv-Script载体(Stratagene,La Jolla,Calif.)、pcDNA4载体(Invitrogen,Carlsbad,Calif.)和pCI-neo载体(Promega,Madison,Wis.)。明显的是，目标基因构建体可以用于引起目标ActRIIB多肽在培养物中增殖的细胞中表达，例如，来产生蛋白质，包括融合蛋白或变体蛋白，用于纯化。

[0290] 用包括一种或更多种目标ActRIIB多肽的编码序列(例如，SEQ ID NO:19或编码可溶ActRIIB多肽的那些核酸序列(例如，编码SEQ ID NO:17、18、23、26、27、29、30、31、32、33、36、37、42和43的核酸))的重组基因转染的宿主细胞可以用于生产适用于本文描述的方法和组合物的ActRIIB多肽。所述宿主细胞可以是任何原核或真核细胞。例如，本文提供的ActRIIB多肽可以在细菌细胞例如E.coli、昆虫细胞(例如，使用杆状病毒表达系统)、酵母或哺乳动物细胞中表达。其他适合的宿主细胞是本领域技术人员公知的。

[0291] 因而，本文提供的是生产用于本文描述的方法和组合物的ActRIIB多肽的方法。例如，用编码ActRIIB多肽的表达载体转染的宿主细胞可以在适当的条件下培养来容许发生ActRIIB多肽的表达。从细胞与含有ActRIIB多肽的培养基的混合物中，可以分泌和分离ActRIIB多肽。做为选择，ActRIIB多肽可以细胞质地或在膜部分中保持，收获细胞、裂解并分离蛋白。细胞培养物包括宿主细胞、培养基和其他副产物。用于细胞培养的适合的培养基是本领域公知的。利用本领域已知的用于纯化蛋白质的技术，可以从细胞培养介质、宿主细胞或两者分离目标ActRIIB多肽，包括离子交换层析、凝胶过滤层析、超滤、电泳、使用特异于ActRIIB多肽的特定表位的抗体的免疫亲和纯化、以及使用结合与ActRIIB多肽融合的结构域的试剂的亲和纯化(例如，蛋白A柱可以用于纯化ActRIIB-Fc融合物)。在优选的实施方式中，ActRIIB多肽是含有便于其纯化的结构域的融合蛋白。在优选的实施方式中，纯化通过一系列柱层析步骤来实现，包括，例如，三种或更多种以下的，按任何顺序：蛋白A层析、Q琼脂糖层析、苯基琼脂糖层析、空间排阻层析和阳离子交换层析。纯化可以用病毒过滤和缓冲液交换来完成。如本文表明的，ActRIIB-hFc蛋白可以纯化到通过空间排阻层折测定的>98％的纯度，和通过SDS-PAGE测定的>95％的纯度。这一水平的纯度足以在小鼠中对骨骼实现期望的效果，以及在小鼠、大鼠和非人灵长类中实现可接受的安全分布。

[0292] 在另一个实施方式中，编码位于重组ActRIIA多肽的期望部分的N-末端的纯化前导序列例如聚-(His)/肠激酶裂解位点序列的融合基因可以容许表达的融合蛋白通过利用Ni2+金属树脂的亲和层析来纯化。纯化前导序列然后通过用肠激酶处理来除去，以提供纯化的ActRIIB多肽(例如，参见Hochuli et al.,(1987)J.Chromatography 411:177;和Janknecht et al.,PNAS USA 88:8972)。

[0293] 制造融合物基因的技术是公知的。基本上，根据常规技术进行编码不同多肽序列的各种DNA片段的连接，采用钝末端的或交错末端的末端用于连接，限制酶消化来提供适当

的末端，视情况填充粘末端，碱性磷酸酶处理来避免不希望的连接，以及酶连接。在另一个实施方式中，融合基因可以通过常规技术，包括自动的DNA合成仪来合成。做为选择，可以使用锚引物来进行基因片段的PCR扩增，所述锚引物产生两个连续基因片段之间的互补的突出，其随后可以退火来产生嵌合基因序列(参见，例如Current Protocols in Molecular Biology,eds.Ausubel et al.,John Wiley&Sons:1992)。

[0294] ActRIIB-Fc融合蛋白可以从pAID4载体(SV40ori/增强子，CMV启动子)在稳定地转染的CHO-DUKX B1 1细胞中表达，使用SEQ ID NO:8的组织血纤蛋白溶酶原前导序列。Fc部分可以包含人IgG1Fc序列，如SEQ ID NO:7所示。在某些实施方式中，在表达时，平均起来，所含的蛋白质具有每分子的ActRIIB-Fc融合蛋白约1.5到2.5摩尔的唾液酸。

[0295] 在某些实施方式中，ActRIIB-Fc融合物的长血清半衰期可以是在人类对象中25-32天。另外，相比在人293细胞中表达的ActRIIB-hFc融合蛋白所报告的，CHO细胞表达的材料可以具有更高的对激活素B配体的亲和力(del Re et al.,J Biol Chem.2004Dec 17;279(51):53126-35)。另外，不受理论的限制，使用比其他前导序列提供更高生产的TPA前导序列，不同于用天然前导区表达的ActRIIB-Fc，可以提供高纯度的N-末端序列。使用天然前导序列可能产生两种主要种类的ActRIIB-Fc，各自具有不同的N-末端序列。

[0296] 8.5.3 其他ACTRII受体信号转导抑制剂

[0297] 在某些实施方式中，本文描述的组合物和方法中使用的ActRII信号转导抑制剂是核酸化合物。

[0298] 抑制ActRII受体的核酸化合物的类别的实例包括反义核酸、siRNA或RNAi构建体以及催化性核酸构建体。核酸化合物可以是单链的或双链的。双链的化合物还可以包括突出的或非互补的区域，其中链的一条或另一条是单链的。单链的化合物可以包括自我互补的区域，意味着所述化合物可以形成所谓的“发夹”或“茎-环”结构，带有双螺旋结构的区域。

[0299] 在某些实施方式中，抑制ActRII受体的核酸化合物可以包含与一区域互补的核苷酸序列，所述区域由全长ActRII受体核酸序列或激活素核酸序列(例如，激活素A或激活素B亚基，也称为βA或βB的核酸序列)的不超过1000个、不超过500个、不超过250个、不超过100个或不超过50、35、30、25、22、20或18个核苷酸组成。在具体的实施方式中，互补的区域将是至少8个核苷酸，任选地至少10个或至少15个核苷酸，任选地在15到25个核苷酸之间。互补的区域可以落入内含子、目标转录产物的编码序列或非编码序列，例如编码序列部分。一般地，抑制ActRII受体的核酸化合物将具有约8个到约500个核苷酸或碱基对的长度，任选地所述长度是约14个到约50个核苷酸。抑制ActRII受体的核酸化合物可以是DNA(特别是作为反义使用的)、RNA或RNA:DNA杂交物。任一条链可以包括DNA和RNA的混合物，以及不容易被分类为DNA或RNA的修饰形式。同样地，双链的核酸化合物可以是DNA:DNA、DNA:RNA或RNA:RNA，任一条链还可以包括DNA和RNA的混合物，以及不容易被分类为DNA或RNA的修饰形式。

[0300] 抑制ActRII受体的核酸化合物可以包括各种修饰的任一种，包括对主干(天然核酸中的糖-磷酸部分，包括核苷酸间连键)或碱基部分(天然核酸的嘌呤或嘧啶部分)的一个或多个修饰。在某些实施方式中，反义核酸化合物将具有约15个到约30个核苷酸的长度，通常将含有一个或更多个修饰来改善某些特性，例如，血清中的稳定性、细胞中的稳定性、化合物可能被递送的部位中的稳定性，例如，就口服递送来说是胃部，以及对于吸入的化合物

来说是肺部。对于RNAi构建体来说，与目标转录产物互补的链一般将是RNA或其修饰体。其他的链可以是RNA、DNA或任何其他的变异体。在某些实施方式中，双链的或单链的“发夹”RNAi构建体的双螺旋部分可以具有18到40个核苷酸的长度，任选地约21到23个核苷酸长度，只要它起到Dicer底物的作用。催化性或酶性核酸可以是核酶或DNA每，也可以含有修饰的形式。在某些实施方式中，在生理条件下以及在无义或正义对照很少作用或没有作用的浓度下，抑制ActRII受体的核酸化合物可以抑制它的目标的表达达到约50％、60％、70％、75％、80％、85％、90％、95％、99％或更多，测试核酸化合物的作用的浓度包括1、5、10微摩尔，或更高。

[0301] 在其他实施方式中，本文描述的组合物和方法中使用的ActRII信号转导抑制剂是抗体。这样的抗体包括结合激活素(特别是激活素A或B亚基，也称为βA或βB)并破坏ActRII受体结合的抗体；以及结合ActRII受体多肽(例如，可溶的ActRIIA或可溶的ActRIIB多肽)并破坏激活素结合的抗体。

[0302] 通过使用衍生自ActRII受体多肽或激活素多肽的免疫原，抗蛋白质/抗肽的抗血清或单克隆抗体可以通过标准方案产生(参见，例如，Antibodies:A Laboratory Manual ed.by Harlow and Lane(Cold Spring Harbor Press:1988))。哺乳动物，例如，小鼠、仓鼠或家兔可以用ActRII受体多肽的免疫原性形式、能够引发抗体反应的抗原性片段或融合蛋白来免疫。对蛋白质或肽赋予免疫原性的技术包括结合到载体，或本领域公知的其他技术。ActRII受体或激活素多肽的免疫原性部分可以在存在佐剂的情况下施用。免疫的进展可以通过检测血浆或血清中的抗体滴度来监视。标准的ELISA或其他免疫分析可以使用免疫原作为抗原来评估抗体的水平。

[0303] 在用ActRII受体多肽的抗原性制品免疫动物之后，可以获得抗血清，如果希望，可以从血清分离多克隆抗体。为了产生单克隆抗体，抗体生产细胞(淋巴细胞)可以从免疫的动物中收获，通过标准的体细胞融合过程与永生化细胞如骨髓瘤细胞融合来产生杂交瘤细胞。这样的技术是本领域公知的，包括，例如，杂交瘤技术(由Kohler and Milstein,(1975)Nature,256:495-497最早开发)、人B细胞杂交瘤技术(Kozbar et al.,(1983)Immunology Today,4:72)以及EBV杂交瘤技术来生产人单克隆抗体(Cole et al.,(1985)Monoclonal Antibodies and Cancer Therapy,Alan R.Liss,Inc.pp.77-96)。可以免疫化学地筛选杂交瘤细胞中与ActRII受体多肽特异性反应的抗体生产，从包含这样的杂交瘤细胞的培养物中分离单克隆抗体。

[0304] 如本文使用的术语“抗体”意图包括也特异性地与目标多肽反应的抗体的片段。抗体可以使用常规技术片段化，按上文对完整抗体描述的相同的方式为了实用性筛选片段。例如，F(ab)2片段可以通过用胃蛋白酶处理抗体产生。产生的F(ab)2片段可以进行处理来还原二硫键，产生Fab片段。抗体进一步意图包括双特异性的、单链的、嵌合的、人源化的和完全的人分子，其具有由抗体的至少一个CDR区赋予的对ActRII受体或激活素多肽的亲和力。抗体可以进一步包含附着于其上并能被检测的标签(例如，所述标签可以是放射性同位素、荧光化合物、酶或酶辅助因子)。

[0305] 在某些实施方式中，抗体是重组抗体，该术语涵盖部分通过分子生物学技术产生的任何抗体，包括CDR移植的或嵌合的抗体、从文库选择的抗体结构域中组装的人抗体或其他抗体、单链抗体和单结构域抗体(例如，人VH蛋白或骆驼化的VHH蛋白)。在某些实施方式

中，抗体可以是单克隆抗体，以及在某些实施方式中。例如，产生特异性结合ActRII受体多肽或激活素多肽的单克隆抗体的方法可以包括，向小鼠施用一定数量的包含有效刺激可检测免疫应答的抗原多肽的免疫原性组合物，从小鼠获得抗体生产细胞(例如，来自脾脏的细胞)，将所述抗体生产细胞与骨髓瘤细胞融合来获得抗体生产杂交瘤，以及测试所述抗体生产杂交瘤来鉴定产生特异性结合所述抗原的单克隆抗体的杂交瘤。一旦获得杂交瘤，杂交瘤可以在细胞培养物中增殖，任选地在杂交瘤衍生的细胞产生特异性结合抗原的单克隆抗体的培养条件下培养。单克隆抗体可以从细胞培养物中纯化。

[0306] 提及抗体时使用的形容词“特异性反应”意图是指，如本领域一般理解的，在目标抗原(例如，ActRII受体多肽)和非目标的其他抗原之间所述抗体是足够有选择性的，使得所述抗体至少在检测特定类型的生物样品中目标抗原的存在方面是有用的。在采用抗体的某些方法例如治疗应用中，更高程度的结合特异性是希望的。单克隆抗体一般具有更高的倾向(相比多克隆抗体)来有效地区分期望的抗原和交叉反应的多肽。影响抗体:抗原相互作用的特异性的一个特征是抗体对抗原的亲和力。虽然可以在大范围的不同亲和力下实现期望的特异性，一般地优选的抗体将具有约10^{-6}、10^{-7}、10^{-8}、10^{-9}或更低的亲和力(解离常数)。考虑到激活素与ActRII受体之间非常紧密的结合，预计的是中和性抗激活素或抗ActRII受体抗体一般应具有10^{-10}或更低的解离常数。

[0307] 此外，用于筛选抗体来鉴定期望的抗体的技术可能影响所获得的抗体的性质。例如，如果抗体用于结合溶液中的抗原，可能希望的是测试溶液结合。多种不同的技术可以用于测试抗体与抗原之间的相互作用来鉴定特别希望的抗体。这样的技术包括ELISA、表面胞质团共振结合分析(例如，Biacore.TM.结合分析，Biacore AB，Uppsala，Sweden)、夹心分析(例如，IGEN International Inc.的顺磁性的珠子系统，Gaithersburg，Md.)、Western印迹、免疫沉淀分析和免疫组织化学。

[0308] 在某些实施方式中，用于本文描述的组合物和方法的ActRII信号转导抑制剂包括可选择形式的激活素，特别是在I型受体结合结构域中具有改变、可以结合II型受体并且不能形成活性的三元复合物的那些。在某些实施方式中，抑制激活素A、B、C或E或特别是ActRII受体表达的核酸，例如，反义分子、siRNA或核酶，可以用于本文描述的组合物和方法中。在某些实施方式中，用于本文描述的组合物和方法的ActRII信号转导抑制剂展现了相比抑制TNF-beta家族的其他成员、抑制GDF11介导的信号转导的选择性，特别是对于GDF8和激活素。

[0309] 在其他实施方式中，本文描述的组合物和方法中使用的ActRII信号转导抑制剂是具有ActRII受体拮抗剂活性的非抗体蛋白，包括抑制素(即，抑制素alpha亚基)、卵泡抑素(例如，卵泡抑素-288和卵泡抑素-315)、Cerberus、卵泡抑素相关蛋白(“FSRP”)、内皮糖蛋白(endoglin)、激活素C、alpha(2)-巨球蛋白和M108A(位置108处甲硫氨酸到丙氨酸的改变)突变的激活素A。

[0310] 在具体的实施方式中，用于本文描述的组合物和方法的ActRII信号转导抑制剂是拮抗激活素生物活性和/或结合激活素的卵泡抑素多肽。术语“卵泡抑素多肽”包括这些多肽，其包含卵泡抑素的任何天然发生的多肽以及保持了有用活性的其任何变体(包括突变体、片段、融合物和肽模拟物形式)，进一步包括卵泡抑素的任何功能单体或多聚体。保持了激活素结合性质的卵泡抑素多肽变体可以根据涉及卵泡抑素和激活素相互作用的早先的

研究来鉴定。例如，WO2008/030367公开了显示对激活素结合重要的特定的卵泡抑素结构域（“FSDs”），通过全部引用将其合并在本文中。卵泡抑素多肽包括一些多肽，其衍生自任何已知的卵泡抑素的序列、具有至少约80％相同于卵泡抑素多肽的序列的序列，任选地至少85％、90％、95％、96％、97％、98％、99％或更高的同一性。卵泡抑素多肽的实例包括成熟的卵泡抑素多肽或较短的同种型或人卵泡抑素前体多肽的其他变体，例如，在WO2005/025601中描述的，通过全部引用将其合并在本文中。

[0311] 在具体的实施方式中，用于本文描述的组合物和方法的ActRII信号转导抑制剂是拮抗激活素生物活性和/或结合激活素的卵泡抑素-样相关基因(FLRG)。术语“FLRG多肽”包括这些多肽，其包含FLRG的任何天然发生的多肽以及保持了有用活性的其任何变体(包括突变体、片段、融合物和肽模拟物形式)保持了激活素结合性质的FLRG多肽变体可以使用分析FLRG和激活素相互作用的常规方法来鉴定。参见，例如，美国专利No.6,537,966，通过全部引用将其包括在本文中。FLRG多肽包括一些多肽，其衍生自任何已知的FLRG的序列、具有至少约80％相同于FLRG多肽的序列的序列，任选地至少85％、90％、95％、96％、97％、98％、99％或更高的同一性。

[0312] 在某些实施方式中，卵泡抑素多肽和FLRG多肽的功能变体或修饰形式包括具有卵泡抑素多肽或FLRG多肽的至少一部分以及一个或更多个融合结构域的融合蛋白，所述融合结构域例如，促进多肽的分离检测或稳定化或多聚化的结构域。上文参考ActRIIA和ActRIIB多肽详细地讨论了适合的融合结构域。在一个实施方式中，ActRII信号转导抑制剂是包含与Fc结构域融合的卵泡抑素多肽的激活素结合部分的融合蛋白。在另一个实施方式中，ActRII信号转导抑制剂是包含与Fc结构域融合的FLRG多肽的激活素结合部分的融合蛋白。

[0313] 8.6 分析

[0314] 各种ActRII多肽变体或可溶的ActRII多肽变体，可以测试它们抑制ActRII的能力。此外，化合物可以测试它们抑制ActRII的能力。一旦确认了ActRII信号转导抑制剂活性，这些化合物可以用于本文提供的方法。ActRII可以是ActRIIA或ActRIIB。以下对ActRIIA描述的分析也可以类似地对ActRIIB进行。

[0315] 8.6.1 评估SNAI1、PHOSPHOSMAD2、PHOSPHOSMAD3、尿蛋白、DKK1、COL1A1、激活素(例如，游离激活素)、RUNX2、ALP、BSAP、CTX、OSTERIX、KLOTHO、ALPHA-SMA、MYOCD、ACTRIIA、AXIN2和/或SM22-ALPHA水平和/或活性

[0316] Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性可以通过本领域已知的或本文描述的任何方法来测定。例如，组织样品中Snai1、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平可以利用例如Northern印迹、PCR分析、实时PCR分析或本领域已知的或本文描述的任何其他技术，通过评估(例如，定量)样品中Snai1、尿蛋白、Dkk1、colla1、激活素、Runx2、Osterix、Alp、BSAP、CTX、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的转录的RNA来测定。本领域技术人员将认识到，Runx2、Alp、Snai1、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2、Sm22-alpha、phosphosmad3和尿蛋白的一种或

更多种的水平和/或活性可以与相应参考群体中的水平和/或活性分别地和独立地比较，例如，如上文在章节8.3.1中描述的。在一个实施方式中，组织样品中Snai1、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平可以分别通过评估(例如，定量)样品中Snai1、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的mRNA来测定。在具体的实施方式中，Snai1、尿蛋白、Dkk1、col1a1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的mRNA水平通过定量逆转录酶聚合酶链式反应(qRT-PCR)来测定。在具体的实施方式中，Runx2mRNA的核酸序列包含SEQ ID NO:58的核酸序列。在具体的实施方式中，Alp mRNA的核酸序列包含SEQ ID NO:59的核酸序列。在具体的实施方式中，Snai1mRNA的核酸序列包含SEQ ID NO:74的核酸序列。在具体的实施方式中，Dkk1mRNA的核酸序列包含SEQ ID NO:75的核酸序列。在具体的实施方式中，col1a1mRNA的核酸序列包含SEQ ID NO:76的核酸序列。在具体的实施方式中，激活素mRNA的核酸序列包含SEQ ID NO:77的核酸序列。在具体的实施方式中，Osterix的核酸序列包含SEQ ID NO:60的核酸序列。在具体的实施方式中，Klotho的核酸序列包含SEQ ID NO:61的核酸序列。在具体的实施方式中，alpha-SMA mRNA的核酸序列包含SEQ ID NO:70的核酸序列。在具体的实施方式中，MYOCD mRNA的核酸序列包含SEQ ID NO:71的核酸序列。在具体的实施方式中，Sm22-alpha mRNA的核酸序列包含SEQ ID NO:62的核酸序列。在具体的实施方式中，使用Runx2特异性引物(SEQ ID NO:48和49)进行qRT-PCR来测定Runx2水平。在具体的实施方式中，使用Alp特异性引物(SEQ ID NO:50和51)进行qRT-PCR来测定Alp水平。在具体的实施方式中，使用Snai1特异性引物(SEQ ID NO:78和79)进行qRT-PCR来测定Snai1水平。在具体的实施方式中，使用Dkk1特异性引物(SEQ ID NO:80和81)进行qRT-PCR来测定Dkk1水平。在具体的实施方式中，使用col1a1特异性引物(SEQ ID NO:82和83)进行qRT-PCR来测定col1a1水平。在具体的实施方式中，使用激活素特异性引物(SEQ ID NO:84和85)进行qRT-PCR来测定激活素水平。在具体的实施方式中，使用Osterix特异性引物(SEQ ID NO:52和53)进行qRT-PCR来测定Osterix水平。在具体的实施方式中，使用Klotho特异性引物(SEQ ID NO:54和55)进行qRT-PCR来测定Klotho水平。在具体的实施方式中，使用Sm22-alpha特异性引物(SEQ ID NO:56和57)进行qRT-PCR来测定Sm22-alpha水平。

[0317] 组织样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平也可以利用例如免疫组织化学分析、Western印迹、ELISA、免疫沉淀、流式细胞计分析或本领域已知的或本文描述的任何其他技术通过分别评估(例如，定量)样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的蛋白表达水平来测定。在具体的实施方式中，Runx2蛋白的氨基酸序列包含SEQ ID NO:63的氨基酸序列。在具体的实施方式中，Alp蛋白的氨基酸序列包含SEQ ID NO:64的氨基酸序列。在具体的实施方式中，Snai1蛋白的氨基酸序列包含SEQ ID NO:86的氨基酸序列。在具体的实施方式中，Dkk1蛋白的氨基酸序列包含SEQ ID NO:87的氨基酸序列。在具体的实施方式中，col1a1蛋白的氨基酸序列包含SEQ ID NO:88的氨基酸序列。在具体的

实施方式中，激活素(例如，游离激活素)蛋白的氨基酸序列包含SEQ ID NO:89的氨基酸序列。在具体的实施方式中，BSAP蛋白的氨基酸序列包含SEQ ID NO:72的氨基酸序列。在具体的实施方式中，Osterix蛋白的氨基酸序列包含SEQ ID NO:65的氨基酸序列。在具体的实施方式中，Klotho蛋白的氨基酸序列包含SEQ ID NO:66的氨基酸序列。在具体的实施方式中，alpha-SMA蛋白的氨基酸序列包含SEQ ID NO:68的氨基酸序列。在具体的实施方式中，MYOCD的氨基酸序列包含SEQ ID NO:69的氨基酸序列。在具体的实施方式中，Sm22-alpha蛋白的氨基酸序列包含SEQ ID NO:67的氨基酸序列。在具体的实施方式中，ActRIIA蛋白的氨基酸序列包含SEQ ID NO:1的氨基酸序列。在具体的实施方式中，ActRIIA蛋白的氨基酸序列包含SEQ ID NO:2的氨基酸序列。在具体的实施方式中，ActRIIA蛋白的氨基酸序列包含SEQ ID NO:3的氨基酸序列。在具体的实施方式中，ActRIIA蛋白的氨基酸序列包含SEQ ID NO:4的氨基酸序列。在具体的实施方式中，ActRIIA蛋白的氨基酸序列包含SEQ ID NO:5的氨基酸序列。在具体的实施方式中，Axin2蛋白的氨基酸序列包含SEQ ID NO:87的氨基酸序列。在特定的实施方式中，Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性通过western印迹来测定。在特定的实施方式中，Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的活性通过Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2或SM22-alpha所调节的蛋白质的western印迹分析来测定。在特定的实施方式中，Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平通过能够分别定量对象的组织样品中(例如，人血清中)存在的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的数量的方法，和/或能够分别检测在用ActRII信号转导抑制剂治疗之后Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平的矫正的方法来测定。在一个实施方式中，组织样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平通过利用ELISA分别评估(例如，定量)样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的蛋白质表达来测定。例如，Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha可以利用ELISA方法在人血清中鉴定和定量。用于测定组织样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平的ELISA方法可以包括用组织样品(例如，人血清)包被ELISA平板，

并分别检测所述组织样品(例如,人血清)中分别结合Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha特异性抗体的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha。在某些实施方式中,如本文描述的用于测定Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性的方法(例如,ELISA)能够检测100pg/ml起的Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha。在具体的实施方式中,用Runx2特异性抗体SC-390715(Santa Cruz)进行ELISA来测定Runx2水平。在具体的实施方式中,用Alp特异性抗体SC-98652(Santa Cruz)进行ELISA来测定Alp水平。在具体的实施方式中,用Snai1特异性抗体sc-393172(Santa Cruz)进行ELISA来测定Snai1水平。在具体的实施方式中,用phosphosmad2特异性抗体sc-101801(Santa Cruz)进行ELISA来测定phosphosmad2水平。在具体的实施方式中,用phosphosmad3特异性抗体sc-130218(Santa Cruz)进行ELISA来测定phosphosmad2水平。在具体的实施方式中,用Dkk1特异性抗体sc-374574(Santa Cruz)进行ELISA来测定Dkk1水平。在具体的实施方式中,用colla1特异性抗体sc-8784(Santa Cruz)进行ELISA来测定colla1水平。在具体的实施方式中,用激活素特异性抗体A1594(Sigma Aldrich)进行ELISA来测定激活素(例如,游离激活素)水平。在具体的实施方式中,用BSAP特异性抗体SC-98652(Santa Cruz)进行ELISA来测定CTX水平。在具体的实施方式中,用CTX特异性抗体ABIN1173415(Antibodies Online)进行ELISA来测定CTX水平。在具体的实施方式中,用Osterix特异性抗体SC-22538(Santa Cruz)进行ELISA来测定Osterix水平。在具体的实施方式中,用Klotho特异性抗体SC-22218(Santa Cruz)进行ELISA来测定Klotho水平。在具体的实施方式中,用alpha-SMA特异性抗体SC-53142(Santa Cruz)进行ELISA来测定alpha-SMA水平。在具体的实施方式中,用MYOCD特异性抗体SC-21561(Santa Cruz)进行ELISA来测定MYOCD水平。在具体的实施方式中,用Sm22-alpha特异性抗体SC-271719(Santa Cruz)进行ELISA来测定Sm22-alpha水平。在具体的实施方式中,用ActRIIA特异性抗体ab 135634(Abcam)进行ELISA来测定ActRIIA水平。

[0318] 本文描述的ELISA分子中描述的Runx2、Alp、Snai1、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2、Sm22-alpha、phosphosmad3和尿蛋白的一种或更多种的水平可以与相应的参考群体中的水平和/或活性独立地和分别地比较,如上文在章节8.3.1中描述的。

[0319] 在测量样品中(例如,组织样品中,例如,主动脉、血液、血清、血浆、肝脏、脾脏和/或骨髓的样品)Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平的分析中使用的抗体是本领域已知的,或可以利用本领域技术人员已知的途径容易地开发。可以用于测量样品中Runx2水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-B4293、LS-B4294、LS-

B4296的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-390715的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:WH0000860M1的抗体。可以用于测量样品中Alp水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-B2877、LS-B1844、LS-C169212的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-98652的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:SAB1405449的抗体。可以用于测量样品中Snail水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-C161335、LS-C198229和LS-C169298的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-393172的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:SAB1404386的抗体。可用于测量样品中phosphosmad2水平的分析中的抗体的实例包括来自Santa Cruz Biotechnology,Inc.,Santa Cruz,CA、目录编号sc-101801的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:SAB4200251和SAB4300252的抗体。可用于测量样品中phosphosmad3水平的分析中的抗体的实例包括来自Santa Cruz Biotechnology,Inc.,Santa Cruz,CA、目录编号sc-130218的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:SAB4300253和SAB4504210的抗体。可以用于测量样品中Dkk1水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-C108793、LS-c105116和LS-C105117的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-374574的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:WH0022943M1的抗体。可用于测量样品中colla1水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-B5932的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-8784的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:C2456的抗体。可用于测量样品中激活素(例如,游离激活素)水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-C308491抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:A1719的抗体。可以用于测量样品中BSAP水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-B2877、LS-B1844和LS-C169212的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-98652的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:SAB1405449的抗体。可用于测量样品中CTX水平的分析中的单克隆抗体的实例包括ABIN1173415(Antibodies Online)。可以用于测量样品中Osterix水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-C139215、LS-C132610、LS-B6531的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-133871的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:WH0121340M1的抗体。可以用于测量样品中Klotho水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-C165587、LS-C145689、LS-C8376的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-22218的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:SAB1306662的抗体。可用于测量样品中alpha-SMA水平的分析中的抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-B6000、LS-B5966、LS-B2161的抗体;可从Santa Cruz

Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号SC-21561的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:A5228的抗体。可用于测量样品中MYOCD水平的分析中的抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-C37407、LS-C153495、LS-c137255的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号SC-53142的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:M8948的抗体。可以用于测量样品中Sm22-alpha水平的分析中的单克隆抗体的实例包括来自LifeSpan Biosciences Inc.,Seattle,WA、目录编号LS-B2563、LS-C139114、LS-C210979的抗体;可从Santa Cruz Biotechnology,Inc.,Santa Cruz,CA获得的、目录编号sc-271719的抗体;以及可从Sigma-Aldrich Co.LLC获得的、产品号码:SAB2501014的抗体。可用于测量样品中ActRIIA水平的分析中的单克隆抗体的实例包括来自Abcam、产品码ab135634的抗体。

[0320] Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的活性可以通过本领域已知的任何分析来测量,非限制性地包括报告基因分析(例如,分别含有Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2或Sm22-alpha响应报告基因构建体),或任何其他生物活性分析。

[0321] Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性可以在获自根据本文描述的方法治疗的对象的任何组织样品中分析。在某些实施方式中,Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平和/或活性在获自根据本文描述的方法治疗的对象的主动脉、血清、肝脏、脾脏或骨髓的样品中评估。在一个实施方式中,Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平和/或活性在获自根据本文描述的方法治疗的对象的血清的样品中评估。在另一个实施方式中,Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平和/或活性在获自根据本文描述的方法治疗的对象的主动脉、脾脏的样品中评估。在一个实施方式中,Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha水平和/或活性在获自根据本文描述的方法治疗的对象的骨髓的样品中评估。

[0322] 本领域技术人员将认识到,利用本领域已知的和本文描述的分析测定的Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2、Sm22-alpha、phosphosmad3和尿蛋白的一种或更多种的活性可以分别与相应参考群体中的水平和/或活性来比较,例如,如上文在章节8.3.1中描述的。

[0323] 在某些实施方式中,对象的组织样品中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、

alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别与如章节8.6中描述的参考群体的组织样品中(例如,来自相同组织的样品中)Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性比较。在某些实施方式中,对象的组织样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别与较早时间点(例如,疾病发作之前、治疗开始之前或在治疗期间)时对象的组织样品中(例如,来自相同组织的样品中)Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性比较。在某些实施方式中,对象的组织样品(例如,主动脉、血清、脾脏、肝脏或血液骨髓)中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别与对象的其他组织样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性比较。在某些实施方式中,对象的组织样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性与对象的组织样品中其他基因产物(例如,b-肌动蛋白、激活素A、激活素B)的水平和/或活性比较。

[0324] 在某些实施方式中,对象的组织样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别与参考群体的组织样品中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性比较。在某些实施方式中,分别与参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性相比、Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的升高的水平和/或活性的检出,继之以施用激活素受体信号转导抑制剂(例如本文描述的一种或更多种激活素受体信号转导抑制剂)。在某些实施方式中,施用激活素受体信号转导抑制剂(例如本文描述的一种或更多种激活素受体信号转导抑制剂)继之以监视Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性,任选地,将所述Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、col1a1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别与参考群体中

Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性比较。在某些实施方式中,施用第一剂量的ActRII信号转导抑制剂(例如,ActRIIA-hFc如SEQ ID NO:7),继之以测定Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性,如果Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的水平和/或活性分别相比参考群体中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的水平和/或活性升高,和/或如果Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别相比参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性降低,施用比所述第一剂量更高(例如,1.25、1.5、1.75、2、2.5、3、4、5、6、7、8、9或10倍高)的第二剂量的ActRII信号转导抑制剂,如果Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的水平和/或活性分别相比参考群体中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的水平和/或活性降低,和/或如果Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别相比参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性升高,施用比所述第一剂量更低(例如,1.25、1.5、1.75、2、2.5、3、4、5、6、7、8、9或10倍低)的第二剂量的ActRII信号转导抑制剂。在某些实施方式中,所述参考群体是章节8.6中描述的群体。

[0325] 在某些实施方式中,治疗的对象的组织样品中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性分别与来自一个或更多个健康对象的相同组织的样品中的Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性比较。在某些实施方式中,评估Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性的组织是主动脉、血清、骨髓、肝脏或脾脏。在一个实施方式中,评估Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性的组织是血清。

[0326] 在某些实施方式中,Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的升高的水平和/或活性分别高于参考群体中Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的水平和/或活性约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、90%、100%、200%或500%;和/或Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的降低的水平分别低于参考群体中Klotho、

alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％。在某些实施方式中，Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的升高的水平和/或活性分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％高于参考群体的前10％、前5％、前4％、前3％、前2％或前1％中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的水平和/或活性；和/或Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的降低的水平分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％低于参考群体的后10％、后5％、后4％、后3％、后2％或后1％中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性。在某些实施方式中，所述参考群体是如章节8.6中描述的。

[0327] 在某些实施方式中，Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的升高的水平和/或活性分别是约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％大于参考群体中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性；和/或Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的降低的水平和/或活性分别是约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％低于参考群体中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的水平和/或活性。在某些实施方式中，Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的升高的水平和/或活性分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％、100％、200％或500％高于参考群体的前10％、前5％、前4％、前3％、前2％或前1％中Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性；和/或Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的降低的水平和/或活性分别等于或约10％、20％、25％、30％、40％、50％、60％、70％、75％、80％、90％或100％低于参考群体的后10％、后5％、后4％、后3％、后2％或后1％中Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的水平和/或活性。在某些实施方式中，所述参考群体是如章节8.6中描述的。

[0328] 在某些实施方式中，激活素的水平是游离激活素的水平，例如，不与如卵泡抑素、卵泡抑素样3或抑制素缔合的激活素。游离激活素的水平可以通过例如(i)定量样品中，例如血浆中激活素的浓度；(ii)定量样品中与激活素缔合的蛋白质，例如卵泡抑素、卵泡抑素样3和抑制素的浓度；和(iii)计算激活素浓度与激活素缔合蛋白的浓度的化学计量比来确定。

[0329] 本文描述的分析还可以用于测定其他的蛋白质和/或转录产物的水平和/或活性，例如，FGF23、卵泡抑素、卵泡抑素样3、抑制素、涉及内皮向间充质转变的蛋白质和/或转录产物。

[0330] 在某些实施方式中，Snai1、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、

激活素(例如,游离激活素)、Runx2、Alp、BSAP、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha的水平和/或活性是组织(例如,组织样品)中的。在某些实施方式中,所述组织是主动脉。在某些实施方式中,所述组织是肾脏。在某些实施方式中,所述组织是骨骼。在某些实施方式中,所述组织是血清。在优选的实施方式中,Runx2、Dkk1、Alp、osterix、sm22-alpha、Klotho、alpha-SMA、ActRIIA、Axin2和MYOCD水平和/或活性是在主动脉中的。在优选的实施方式中,phosphosmad2、phosphosmad3、尿蛋白、ActRIIA、Axin2和col1a1的水平和/或活性是在肾脏中的。在优选的实施方式中,所述激活素水平和/或活性是在血清中的。在某些实施方式中,所述升高的激活素水平是在管周肌成纤维细胞中的。在某些实施方式中,所述升高的激活素水平不是在肾脏上皮中的。在某些实施方式中,所述升高的激活素水平是在管周肌成纤维细胞中的,并且所述升高的激活素水平不是在肾脏上皮中的。

[0331] 本领域技术人员将认识到,Runx2、Alp、Snail、phosphosmad2、Dkk1、col1a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2、Sm22-alpha、phosphosmad3和尿蛋白的一种或更多种的水平和/或活性可以与相应参考群体中的水平和/或活性分别地和独立地比较,例如,如上文在章节8.3.1中描述的。

[0332] 8.6.2 参考群体

[0333] 在某些实施方式中,利用获自本文描述的参考群体的数据(例如,生物标记物水平或临床症状)来确定从根据本文提供的方法治疗的或要治疗的对象获得的类似数据是病理学上高的(例如,提高的)还是低的(例如,降低的)。

[0334] 在某些实施方式中,参考群体的大小可以是1、5、10、25、50、75、100、200、250、300、400、500或1000个个体。在某些实施方式中,所述参考群体由随机的志愿者组成。在某些实施方式中,所述参考群体由健康人组成。在某些实施方式中,所述参考群体由与章节8.4中描述的患者群体相同年龄、体重和/或性别的人组成。在某些实施方式中,所述参考群体由没有心血管疾病的人组成。在某些实施方式中,所述参考群体由没有血管钙化的人组成。在某些实施方式中,所述参考群体由没有心血管疾病的人组成。在某些实施方式中,所述参考群体由没有与血管钙化相关和/或由血管钙化引起的心血管疾病的人组成。在某些实施方式中,所述参考群体由没有肾病的人组成。在某些实施方式中,所述参考群体由没有慢性肾病的人组成。在某些实施方式中,所述参考群体由没有动脉硬化水平病理性升高的人组成。在某些实施方式中,所述参考群体由没有与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病的人组成。在某些实施方式中,所述参考群体由没有LVH的人组成。在某些实施方式中,所述参考群体由没有与LVH相关和/或由LVH引起的心血管疾病的人组成。

[0335] 在某些实施方式中,所述参考群体是指在心血管疾病的一种或更多种症状发作之前根据本文提供的方法治疗的对象。在某些实施方式中,所述参考群体是指在血管钙化的一种或更多种症状发作之前根据本文提供的方法治疗的对象。在某些实施方式中,所述参考群体是指在慢性肾病的一种或更多种症状发作之前根据本文提供的方法治疗的对象。在某些实施方式中,所述参考群体是指在动脉硬化水平升高的一种或更多种症状发作之前根据本文提供的方法治疗的对象。在某些实施方式中,所述参考群体是指在LVH的一种或更多种症状发作之前根据本文提供的方法治疗的对象。在某些实施方式中,所述参考群体是指

在诊断对象的心血管疾病之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的血管钙化之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的慢性肾病之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的1期慢性肾病之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的2期慢性肾病之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的3期慢性肾病之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的4期慢性肾病之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的5期慢性肾病之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的动脉硬化水平升高之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体是指在诊断对象的LVH之前根据本文提供的方法治疗的对象。在某些实施方式中，所述参考群体由在根据本文提供的方法治疗之前或被诊断患有本文描述的疾病之前显示了动脉硬化提高的人组成。在某些实施方式中，所述参考群体由在根据本文提供的方法治疗之前或被诊断患有本文描述的疾病之前显示了概述(参见章节6)中记载的标志物之一的上升倾向的人组成。在某些实施方式中，所述参考群体由在根据本文提供的方法治疗之前或被诊断患有本文描述的疾病之前显示了提高水平的Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、Osterix、phosphosmad3和/或尿蛋白的人组成。在某些实施方式中，所述参考群体由在根据本文提供的方法治疗之前或被诊断患有本文描述的疾病之前显示了概述(参见章节6)中记载的标志物之一的下降倾向的人组成。在某些实施方式中，所述参考群体由在根据本文提供的方法治疗之前或被诊断患有本文描述的疾病之前显示了降低水平的alpha-SMA、MYOCD、Sm22-alpha或ActRIIA的人组成。

[0336] 8.6.3 内皮-间充质转变

[0337] 在某些实施方式中，根据本文提供的方法治疗的对象中的EnMT可以通过谱系跟踪分析来监视，以确定细胞群体的结局。在某些实施方式中，根据本文提供的方法治疗的对象中的EnMT可以通过定量与EnMT相关的一种或更多种蛋白质或转录产物的水平和/或活性来监视，例如，Snail。

[0338] 8.6.4 骨周转

[0339] 骨周转的各种循环标志物可以用于诊断骨失调，例如，低骨周转。骨周转的循环标志物是骨形成的标志物，例如，骨特异性碱性磷酸酶(bAP)、osteocalcin、前胶原I型C-末端原肽(PICP)和胰岛素样生长因子-1(IGF-1)，一些是骨吸收的标志物，例如，吡啶啉、脱氧吡啶啉、酒石酸抗性酸性磷酸酶(TRAP)、5b型TRAP、吡啶啉、脱氧吡啶啉和前胶原I型C-末端端肽(ICTP)，血清或尿胶原蛋白交联(N-端肽或C-端肽)和25羟基维生素D。也可以使用测量整个甲状旁腺激素(PTH)分子的分析。熟练的技术人员了解容许评估骨矿物密度(BMD)、骨体积、小梁骨体积和小梁厚度的成象方法。参见，例如Tilman B.Drueke and Sharon M.Moe, Disturbances of bone and mineral metabolism in chronic kidney disease:an international initiative to improve diagnosis and treatment,Nephrol Dial Transplant(2004)19:534-536;Okuno S,Inaba M.,Biochemical markers of bone turnover.New aspect.Dialysis and bone metabolic marker,Clin Calcium.2009 Aug;

19(8):1084-91;Herberth J,Monier-Faugere MC,Mawad HW,Branscum AJ,Herberth Z,Wang G,Cantor T,Malluche HH,The five most commonly used intact parathyroid hormone assays are useful for screening but not for diagnosing bone turnover abnormalities in CKD-5subjects,Clin Nephrol.2009 Jul;72(1):5-14;Lehmann G,Ott U,Kaemmerer D,Schuetze J,Wolf G.,Bone histomorphometry and biochemical markers of bone turnover in subjects with chronic kidney disease Stages 3-5,Clin Nephrol.2008Oct;70(4):296-305;Drüeke TB.,Is parathyroid hormone measurement useful for the diagnosis of renal bone disease?,Kidney Int.2008Mar;73(6):674-6;Yamada S,Inaba M,Kurajoh M,Shidara K,Imanishi Y,Ishimura E,Nishizawa Y.,Utility of serum tartrate-resistant acid phosphatase (TRACP5b)as a bone resorption marker in subjects with chronic kidney disease: independence from renal dysfunction.,Clin Endocrinol(Oxf).2008Aug;69(2):189-96.Epub 2008 Jan 23。还参见,Paul D.Miller,Diagnosis and Treatment of Osteoporosis in Chronic Renal Disease,2009。

[0340] 监视患有轻微的肾脏功能障碍的CKD对象中骨吸收的另一个标志物是I型胶原蛋白N-端肽(S-NTX)的血清浓度。参见,例如Hamano T,Fujii N,Nagasawa Y,Isaka Y,Moriyama T,Okada N,Imai E,Horio M,Ito T.,Serum NTX is a practical marker for assessing antiresorptive therapy for glucocorticoid treated subjects with chronic kidney disease.,Bone.2006 Nov;39(5):1067-72.Epub 2006 Jun 16。

[0341] 定量的计算机断层扫描(QCT)也可以用于测定骨周转。

[0342] 可以评估标志物,例如Runx2和Alp,来监视对象中的成骨细胞转变。可以评估标志物,例如Sm22-alpha,来监视血管平滑肌功能和分化的血管平滑肌细胞的水平。

[0343] 8.6.5 钙水平

[0344] 可以通过本领域技术人员已知的方法,例如,钙离子选择电极来分析钙水平。在某些实施方式中,总钙水平是在血清、血液、主动脉或尿液中测量的。

[0345] 8.6.6 血管钙化

[0346] 用于成像冠状动脉钙化程度(CAC)的非对比计算机断层扫描(CT)和用于非侵入性冠状动脉造影(CTA)的对比CT是通常用于诊断阻塞性冠心病的发展。也可以使用用于诊断性和预后性心脏评估的放射性核素强度测试、冠状动脉钙扫描和非侵入性冠状动脉血管造影。参见:Berman DS,Shaw LJ,Hachamovitch R,Friedman JD,Polk DM,Hayes SW,Thomson LE,Germano G,Wong ND,Kang X,Rozanski A.,Comparative use of radionuclide stress testing,coronary artery calcium scanning,and noninvasive coronary angiography for diagnostic and prognostic cardiac assessment,Semin Nucl Med.2007 Jan;37(1):2-16。

[0347] 来自无症状对象的冠状动脉钙筛查结果可以用作比较。例如,当血管钙化与肾病相关时,在肾病发作之前获得的钙筛查结果可以用作比较。

[0348] 用于检测和定量冠状动脉钙化(CAC)的可能的方法包括,但不限于,X-射线计算机断层扫描和心肌灌流单光子发射计算机断层照相(SPECT)。Moser KW,O'Keefe JH Jr,Bateman TM,McGhie IA.,Coronary calcium screening in asymptomatic subjects as

a guide to risk factor modification and stress myocardial perfusion imaging,J Nucl Cardiol.2003 Nov-Dec;10(6):590-8。多检测器计算机断层扫描(MDCT)也可以用于检测血管钙化(参见,例如,Burrill et al.,2007,Postgrad.Med.J.83(985):698-704)。

[0349] 血管钙化的另一种诊断方法是联合正电子发射断层扫描(PET)/计算机断层扫描(CT)在胸主动脉壁中的氟18氟脱氧葡萄糖(FDG)摄取。参见:Tatsumi M,Cohade C,Nakamoto Y,Wahl RL.,Fluorodeoxyglucose uptake in the aortic wall at PET/CT:possible finding for active atherosclerosis,Radiology.2003 Dec;229(3):831-7.Epub 20030ct 30。

[0350] 在再另一个实施方式中,超快速CT可以用于检测动脉粥样硬化性冠心病的存在。参见,例如Breen JF,Sheedy PF 2nd,Schwartz RS,Stanson AW,Kaufmann RB,Moll PP,Rumberger JA,Coronary artery calcification detected with ultrafast CT as an indication of coronary artery disease,Radiology.1992 Nov;185(2):435-9。

[0351] 电子束计算机断层扫描也可以用于诊断冠状动脉病。参见:Schmermund A,Baumgart D,Sack S,Möhlenkamp S,Grönemeyer D,Seibel R,Erbel R.,Assessment of coronary calcification by electron-beam computed tomography in symptomatic subjects with normal,abnormal or equivocal exercise stress test,Eur Heart J.2000 Oct;21(20):1674-82。

[0352] 血管钙化的另一个测试涉及致丛性和血栓栓塞性肺动脉高压中的斑块组成。慢性血栓栓塞性肺动脉高血压与带有富含血型糖蛋白的松软核心的动脉粥样硬化斑块相关,致丛性肺动脉高血压与纤维状斑块相关。血栓栓塞材料在松软核心的形成中起到关键作用,其中红血球膜衍生的血型糖蛋白是主要成分。因而,研究了慢性血栓栓塞性和致丛性肺动脉高血压(原发性和继发性(Eisenmenger综合征))。参见:Arbustini E,Morbini P,D'Armini AM,Repetto A,Minzioni G,Piovella F,ViganóM,Tavazzi L,Plaque composition in plexogenic and thromboembolic pulmonary hypertension:the critical role of thrombotic material in pultaceous core formation,Heart.2002 Aug;88(2):177-82。

[0353] Agatston评分,一种基于沉积的钙斑块的密度测定的钙评分系统,可以用于定量血管钙化。在这一系统中,血管钙化的水平可以通过多检测器计算机断层扫描(MDCT)来测量,可以评估Agatston评分中进展速率的减弱。(参见,例如Sharma et al.,2010,Vasc.Health Risk Manag.6:603-611)。

[0354] 进一步的,血管钙化可以使用Adragao et al.,2004,Nephrol.Dial.Transplant 19:1480-1488中描述的方法评估。

[0355] 用于定量对象中的血管钙化的另一种分析是病变特异性钙评分,其包括由冠状动脉钙化CT检查产生的钙测量方法。这种方法由例如Akram and Voros,2008,Int.J.cardiovac.Imaging 14:743-749描述。

[0356] 8.6.7 心脏大小和心脏肥大

[0357] 心脏大小和心脏肥大可以通过熟练的技术人员已知的任何方法来测定,例如,磁共振成象、心电图描记术、超声波心动描记术和非对比增强的心脏计算机断层扫描。

[0358] 8.6.8 动脉硬化

[0359] 动脉硬化的水平可以通过熟练的技术人员已知的任何方法来测量，例如，超声多普勒测试，包括磁共振动脉造影的磁共振成像，包括CT血管造影的计算机断层扫描(CT)和本领域已知的其他血管造影形式。

[0360] 8.6.9 肾脏疾病

[0361] 肾小球滤过率、菊糖清除率、高磷酸血症和BUN水平可以通过本领域技术人员已知的任何方法来测定，以确定肾脏疾病。肾脏纤维化和/或肾小球硬化症可以通过熟练的技术人员已知的任何方法诊断和/或监视，例如，肾脏组织的活检以及组织瘢痕形成检查。肾脏纤维化和/或肾小球硬化症还可以通过例如测量肾小球过滤率和/或进行肾脏超声来诊断和/或监视。参见国家的肾脏基金会的网站。

[0362] 8.6.10 动物模型

[0363] 动脉粥样硬化性低密度脂蛋白受体缺陷(ldlr-/-)的雄性(C57Bl/6J背景)可以购自Jackson实验室，在12周龄开始用高脂肪饮食(42％的卡路里来自脂肪)(Teklad#_)饲喂。小鼠在22周龄时是肥胖的、胰岛素抗性的，在28周龄时是糖尿病的，并且是高胆固醇血症的。

[0364] 早先描述了用来产生慢性肾病的两步过程(Davies,M.R.,et al.,2003.J Am Soc Nephrol 14:1559-1567;Davies,M.R.,et al.,2005.J Am Soc Nephrol 16:917-928.)。电灼可以在出生后12周通过2厘米的侧腹切口施用于右肾，随后在14周龄左侧进行全肾切除术。改变烧灼的强度，以产生中度的(CKD-3)肾脏损伤，其通过20周龄时的菊糖清除率来确认。对照组的小鼠，野生型C57Bl/6J小鼠饲喂常规的饮食，其是用于使对照值标准化的正常肾功能和饮食的组。第二组是饲喂高脂肪饮食和假操作的ldlr-/-小鼠，其具有正常的肾脏功能，充当对照组来测定肾脏疾病的作用。第三个组是具有相当于人CKD 3期的降低的GFR、饲喂高脂肪饮食(CKD-3)的ldlr-/-小鼠，在22周安乐死，是基线血管钙化组(CKD-3)。第四个组是在22周开始每周两次接受溶媒的皮下注射的患有CKD-3的ldlr-/-小鼠，直到第28周安乐死(CKD-3 V)。第五个组是在22周开始每周两次接受10mg/kg的mActRIIA-Fc(Celgene,Summit,NJ)的皮下注射的患有CKD-3的ldlr-/-小鼠，直到第28周安乐死(CKD-3 mActRIIA-Fc)。使用的剂量是早先在PK/PD研究中对于刺激骨形成有效的剂量(Lotinun,S.,et al.,2010.Bone46:1082-1088.)。

[0365] 使用的第二个CKD的模型是X-连锁的Alport's综合征的鼠同源物，其在IV型胶原蛋白的α5链的基因上是缺陷的，COL4A5(Rheault,M.N.,et al.,2004.Journal of the American Society of Nephrology 15:1466-1474.)。这是自发性肾脏疾病的模型。繁育对可以购自Jackson实验室，并繁育用于实验。半合子雄性在出生后200天自发地发生与人3-4期CKD可比较的肾脏疾病。

[0366] 第三个CKD模型是在用于细胞谱系追踪的转基因小鼠GNZ小鼠中的肾脏消融，类似于ldlr-/-方案。GNZ报告物雌性小鼠()和Tek-Cre转基因雄性小鼠()可以购自Jackson实验室，并繁育产生GNZ/Tek-Cre^{+}小鼠用于实验。GNZ/Tek-Cre-同窝出生作为阴性对照。可以通过使用厂家推荐用于GNZ和Tek-Cre小鼠品系的特异性引物进行小鼠基因分型。

[0367] 8.6.11 转录反应分析

[0368] 在某些实施方式中，转录反应分析可以用于测试ActRII信号转导抑制剂或Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、

Alp、BSAP、CTX、Osterix、Klotho、ActRIIA、Axin2和/或Sm22-alpha的活性。在ActRII、Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Osterix、Alp、BSAP、CTX、Klotho、ActRIIA、Axin2和/或Sm22-alpha信号转导时,某些基因的转录被上调或下调。可以测量使用的细胞培养系统和转录反应(例如,通过RT-PCR)。试剂对转录反应的影响是其有效性或活性的度量。在某些实施方式中,已知对ActRII、Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如,游离激活素)、Runx2、Osterix、Alp、BSAP、CTX、Klotho、ActRIIA、Axin2和/或Sm22-alpha信号转导响应的启动子区域可以被克隆到报告基因的上游。这样,可以简化分析,从而仅需要分析报告基因的活性。

[0369] 8.6.12 筛选分析

[0370] 各种ActRII多肽变体或可溶的ActRII多肽变体,可以测试它们抑制ActRII的能力。此外,化合物可以测试它们抑制ActRII的能力。一旦确认了ActRII信号转导抑制剂活性,这些化合物可以用于本文提供的方法。ActRII可以是ActRIIA或ActRIIB。以下对ActRIIA描述的分析也可以类似地对ActRIIB进行。

[0371] 例如,可以评估ActRIIA多肽变体对涉及骨生产或骨破坏的基因的表达的影响。根据需要,这可以在存在一种或更多种重组的ActRIIA配体蛋白(例如,激活素)的情况下进行,细胞可以被转染,以产生ActRIIA多肽和/或其变体,任选地,产生ActRIIA配体。同样地,ActRIIA多肽可以施用给小鼠或其他动物,可以评估一种或更多种骨性质,例如密度或体积。还可以评估骨折的治愈率。双能X射线吸收测定法(DEXA)是一种用于评估动物骨密度的成熟的非侵入性定量技术。在人类中,中枢DEXA系统可以用于评估脊柱和骨盆的骨密度。这些是总体骨密度的最佳预测值。外周DEXA系统可以用于评估外周骨中的骨密度,包括,例如,手、腕部、踝和脚的骨骼。传统的X射线成像系统,包括CAT扫描,可以用于评估骨生长和骨折愈合。此外,骨密度可以使用定量计算机断层扫描(qCT)来测量。还可以评估骨的机械强度。

[0372] 在某些方面,本文提供的是ActRIIA多肽(例如,可溶的ActRIIA多肽)和激活素多肽鉴定作为激活素-ActRIIA信号转导途径的激动剂或拮抗剂的化合物(试剂)的用途。可以测试通过这种筛选鉴定的化合物,来评估它们在体外调节骨生长或矿化的能力。任选地,这些化合物可以进一步在动物模型中测试,来评估它们在体内调节组织生长的能力。

[0373] 有许多方法来筛选通过靶向激活素和ActRIIA多肽调节组织生长的治疗试剂。在某些实施方式中,可以进行化合物的高通量筛选,来鉴定干扰激活素或ActRIIA介导的骨影响的试剂。在某些实施方式中,进行分析来筛选或鉴定特异性抑制或降低ActRIIA多肽与激活素的结合的化合物。做为选择,所述分析可以用于鉴定增强ActRIIA多肽与激活素的结合的化合物。在进一步的实施方式中,可以通过与激活素或ActRIIA多肽相互作用的能力来鉴定化合物。

[0374] 多种分析形式将是足够的,根据当前的公开,本文没有明确描述的那些仍然可被本领域普通技术人员理解。如本文描述的,本文使用的测试化合物(试剂)可以通过任何组合化学方法来创造。做为选择,目标化合物可以是体内或体外合成的天然发生的生物分子。例如,要测试其作为组织生长调节物的能力的化合物(试剂)可以通过例如细菌、酵母、植物或其他生物体(例如,天然产物)产生,化学地产生(例如,小分子,包括肽模拟物),或重组地

产生。本文期待的测试化合物包括非肽基有机分子、肽、多肽、肽模拟物、糖类、激素和核酸分子。在具体的实施方式中，测试试剂是具有小于约2000道尔顿分子量的小的有机分子。

[0375] 测试化合物可以作为单个的、离散的实体提供，或在更大复杂性的文库中提供，例如，通过组合化学作用制备。这些文库可以包含，例如，醇类、卤烃类、胺类、酰胺类、酯类、醛类、醚类和其他类别的有机化合物。测试化合物向测试系统的呈现可以是分离的形式，或作为化合物的混合物，特别是在初步的筛选步骤中。任选地，化合物可以用其他化合物衍生化，并具有便于化合物分离的衍生基团。衍生基团的非限制性实例包括生物素、荧光素、地加氧原素、绿色荧光蛋白、同位素、多组氨酸、磁珠、谷胱甘肽S转移酶(GST)、光活化性交联剂或其任何组合。

[0376] 在测试化合物和天然提取物的库的许多药物筛选程序中，高通量分析是期望的，以最大化在给定时间内调查的化合物的数量。在无细胞系统中进行、例如可以用纯化的或半纯化的蛋白质衍生化的分析，通常优选的作为“初级”筛选，因为它们可以被产生以允许快速开发和相对容易地检测由测试化合物介导的分子目标中的改变。此外，测试化合物的细胞毒性和/或生物利用率的影响一般可以在体外系统中忽视，该分析反而主要集中于药物对分子目标的影响，在ActRIIA多肽和激活素之间结合亲和性的改变方面这可能是明显的。

[0377] 仅为了说明，在示范性的筛选分析中，感兴趣的化合物与分离的和纯化的ActRIIA多肽接触，所述ActRIIA多肽通常能够结合激活素。然后向化合物和ActRIIA多肽的混合物中添加含有ActRIIA配体的组合物。ActRIIA/激活素复合物的检测和定量提供了一种手段来测定化合物抑制(或强化)ActRIIA多肽与激活素之间复合物形成的能力。化合物的效力可以通过从使用各种浓度的测试化合物获得的数据产生剂量反应曲线来评定。此外，还可以进行对照分析来提供用于对比的基线。例如，在对照分析中，分离的和纯化的激活素被添加到含有ActRIIA多肽的组合物中，在不存在测试化合物的情况下测定ActRIIA/激活素复合物的形成。要理解的是，一般地，混合反应物的顺序可以变化，可以同时地混合。此外，代替纯化的蛋白质，细胞提取物和溶胞产物可以用于实现适合的无细胞分析系统。

[0378] ActRIIA多肽与激活素之间的复合物形成可以通过多种技术来检测。例如，使用例如可检测地标记的蛋白质，例如放射性标记的(例如，^{32}P、^{35}S、^{14}C或^{3}H)、荧光标记的(例如，FITC)或酶标记的ActRIIA多肽或激活素，通过免疫分析，或通过层析检测，可以测定复合物形成的调节。

[0379] 在某些实施方式中，在直接地或间接地测量ActRIIA多肽与它的结合蛋白之间的相互作用程度时，本文期待的是使用荧光偏振分析和荧光共振能量传递(FRET)分析。进一步的，其他检测方式，例如，基于光波导(PCT公开WO 96/26432和美国专利No.5,677,196)、表面胞质团共振(SPR)、表面电荷传感器和表面力传感器的那些，与本文描述的许多实施方式是相容的。

[0380] 此外，相互作用陷阱分析，也称为“双杂交分析”可以用于鉴定破坏或强化ActRIIA多肽与它的结合蛋白之间的相互作用的试剂。参见，例如，美国专利No.5,283,317;Zervos et al.(1993)Cell 72:223-232;Madura et al.(1993)J Biol Chem 268:12046-12054;Bartel et al.(1993)Biotechniques 14:920-924;和Iwabuchi et al.(1993)Oncogene 8:1693-1696)。在具体的实施方式中，本文期待的是使用反转的双杂交系统来鉴定解除

ActRIIA多肽与它的结合蛋白之间的相互作用的化合物(例如,小分子或肽)。参见,例如Vidal and Legrain,(1999)Nucleic Acids Res 27:919-29;Vidal and Legrain,(1999)Trends Biotechnol 17:374-81;和美国专利Nos.5,525,490;5,955,280;和5,965,368。

[0381] 在某些实施方式中,通过它们与ActRIIA或激活素多肽相互作用的能力来鉴定出目标化合物。化合物与ActRIIA或激活素多肽之间的相互作用可以是共价的或是非共价的。例如,这样的相互作用可以使用体外的生物化学方法在蛋白质水平上鉴定,包括光交联、放射性标记的配体结合和亲和层析(Jakoby W B et al.,1974,Methods in Enzymology 46:1)。在某些情况下,化合物可以在基于机制的分析中筛选,例如,检测与激活素或ActRIIA多肽结合的化合物的分析。这可以包括固相或液相的结合事件。做为选择,编码激活素或ActRIIA多肽的基因可以与报告物系统(例如,β-半乳糖苷酶、荧光素酶或绿色荧光蛋白)一起转染到细胞中,并优选地通过高通量筛选针对文库来筛选,或用文库的独立成员来筛选。可以使用其他基于机制的结合分析,例如,检测自由能变化的分析。结合分析可以用固定在反应孔、珠子或芯片上或通过固定化抗体捕获的或通过毛细管电泳分辨的靶标来进行。结合的化合物通常可以使用比色或荧光或表面胞质团共振来检测。

[0382] 在某些方面,本文提供的是调节(刺激或抑制)骨形成和提高骨质量的方法和试剂。因而,所鉴定的任何化合物可以在完整细胞或组织中体外或体内地测试,来确认它们调节骨生长或矿化的能力。为此目标可以利用本领域已知的各种方法。特别地,化合物可以测试它们提高骨周转的能力。

[0383] 例如,ActRIIA或激活素多肽或测试化合物对骨或软骨生长的影响,可以通过在基于细胞的分析中测量Msx2的诱导或骨祖细胞向成骨细胞的分化来测定(参见,例如Daluiski et al.,Nat Genet.2001,27(1):84-8;Hino et al.,Front Biosci.2004,9:1520-9)。基于细胞的分析的其他实例包括分析目标ActRIIA或激活素多肽以及测试化合物在间充质祖代和成骨细胞中的成骨活性。举例来说,可以构建表达激活素或ActRIIA多肽的重组腺病毒,来感染多能的间充质祖代C3H10T1/2细胞、前成骨细胞性C2C12细胞以及成骨细胞性TE-85细胞。然后通过测量碱性磷酸酶的诱导、骨钙素和基质矿化来确定成骨活性(参见,例如Cheng et al.,J bone Joint Surg Am.2003,85-A(8):1544-52).

[0384] 本文还提供的是测量骨或软骨生长的体内分析。例如,公开了Namkung-Matthai et al.,Bone,28:80-86(2001)大鼠骨质疏松模型,在其中研究了骨折后早期的骨修复。Kubo et al.,Steroid Biochemistry&Molecular Biology,68:197-202(1999)也公开了大鼠骨质疏松模型,在其中研究了骨折后早期的骨修复。描述了小鼠骨质疏松症模型,其中小鼠是切除卵巢的,这引起小鼠丢失实质的骨矿物含量以及骨矿物密度,其小梁骨损失大约50%的骨矿物密度。通过施用因子例如甲状旁腺激素,切除卵巢的小鼠中的骨密度可以提高。在某些方面,可以使用本领域已知的骨折愈合分析。这些分析包括骨折技术、组织学分析和生物力学分析,其在例如美国专利No.6,521,750中描述了,对于它引起以及测量骨折的程度和修复过程的实验方案公开内容,通过将其完全引用合并在本文中。

[0385] 8.7 激活素II型受体信号转导抑制剂的剂量

[0386] 在某些实施方式中,ActRII信号转导抑制剂是章节8.5.1中阐述的ActRIIA信号转导的抑制剂。在其他实施方式中,ActRII抑制剂是章节8.5.2中阐述的ActRIIB信号转导的抑制剂。在某些实施方式中,ActRII信号转导抑制剂是ActRIIA信号转导抑制剂与ActRIIB

信号转导抑制剂的组合。

[0387] 在某些实施方式中，ActRII信号转导抑制剂以足以实现0.2微克/kg或更高的血清浓度的间隔和数量给药，1微克/kg或2微克/kg或更高的血清水平对于实现对骨密度和强度的显著影响是希望的。可以设计给药方案来达到0.2到15微克/kg之间的血清浓度，任选的1到5微克/kg之间。在人类中，0.2微克/kg的血清水平可以用0.1mg/kg或更高的单次剂量来实现，1微克/kg的血清水平可以用0.3mg/kg或更高的单次剂量来实现。观察到的分子血清半衰期在约20到30天之间，实质上更长于大多数Fc融合蛋白，因而可以实现持续有效的血清水平，例如，通过用0.2-0.4mg/kg每周或每两周给药，或者更高的剂量可以与给药之间更长的间隔一起运用。例如，可以在每月或每两个月的基础上使用1-3mg/kg的剂量，对骨的作用可能足够耐久，从而仅需要每3、4、5、6、9、12或更多个月给药一次。ActRII信号转导抑制剂的血清水平可以通过熟练的技术人员已知的任何手段来测量。例如，利用例如ELISA，针对ActRII信号转导抑制剂的抗体可以用于测定ActRII信号转导抑制剂的血清水平。

[0388] 在某些实施方式中，ActRII信号转导抑制剂的剂量静脉内地是0.01到3.0mg/kg，或皮下地是0.03到0.1mg/kg。在某些实施方式中，ActRII信号转导抑制剂的剂量是约0.01mg/kg、约0.1mg/kg、约0.13mg/kg、约0.2mg/kg、约0.26mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.5mg/kg、约2.0mg/kg、约2.5mg/kg、约3.0mg/kg、约3.5mg/kg、约4.0mg/kg、约4.5mg/kg或约5.0mg/kg。在某些实施方式中，ActRII信号转导抑制剂的剂量是约10.0mg/kg、约15.0mg/kg、约20.0mg/kg、约25.0mg/kg或约30.0mg/kg。在某些实施方式中，ActRII信号转导抑制剂的剂量是在0.01mg/kg到0.1mg/kg之间、0.1mg/kg到0.3mg/kg之间、0.3mg/kg到0.5mg/kg之间、0.3mg/kg到0.8mg/kg之间、0.5mg/kg到1.0mg/kg之间、1.0mg/kg到2.0mg/kg之间、1.0mg/kg到3.0mg/kg之间、2.0mg/kg到3.0mg/kg之间、2.0mg/kg到4.0mg/kg之间、3.0mg/kg到5.0mg/kg之间、5.0mg/kg到10.0mg/kg之间、10.0mg/kg到15.0mg/kg之间、10.0mg/kg到20.0mg/kg之间、15.0mg/kg到20.0mg/kg之间或20.0mg/kg到30.0mg/kg之间。在某些实施方式中，所述剂量是约15mg、约30mg、约45mg、约60mg、约75mg、约90mg或约1g。在某些实施方式中，所述剂量是约0.1mg/kg。在某些实施方式中，所述剂量是约0.3mg/kg。在某些实施方式中，所述剂量是约0.5mg/kg。在某些实施方式中，所述剂量是约0.7mg/kg。

[0389] 在某些实施方式中，所述剂量是药学上有效的剂量。在某些实施方式中，所述药学上有的效剂量是约15mg、约30mg、约45mg、约60mg、约75mg、约90mg或约1g或约0.1mg/kg、约0.13mg/kg、约0.2mg/kg、约0.26mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg。在某些实施方式中，所述药学上有效的剂量是约0.1mg/kg。在某些实施方式中，所述药学上有效的剂量是约0.3mg/kg。在某些实施方式中，所述药学上有效的剂量是约0.5mg/kg。在某些实施方式中，所述药学上有效的剂量是约0.7mg/kg。

[0390] 在某些实施方式中，所述剂量是初始剂量。在某些实施方式中，所述初始剂量是约15mg、约30mg、约45mg、约60mg、约75mg、约90mg或约1g或约0.1mg/kg、约0.13mg/kg、约0.2mg/kg、约0.26mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg。在某些实施方式中，所述药学上有效的剂量是约0.1mg/kg。在某些实施方式

中，所述初始剂量是约0.3mg/kg。在某些实施方式中，所述初始剂量是约0.5mg/kg。在某些实施方式中，所述初始剂量是约0.7mg/kg。在某些实施方式中，所述初始剂量是(i)每28天施用一次；或(ii)每42天施用一次。在某些实施方式中，所述初始剂量是每14天施用一次。在某些实施方式中，所述初始剂量是每21天施用一次。

[0391] 在某些实施方式中，所述初始剂量每1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27或28天施用一次。在某些实施方式中，所述初始剂量每1、2、3、4、5或6周施用一次。在某些实施方式中，所述初始剂量每2周施用一次。在某些实施方式中，所述初始剂量是约0.1mg/kg、约0.13mg/kg、约0.2mg/kg、约0.26mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg，并且每1、2、3、4、5或6周施用一次。在某些实施方式中，所述初始剂量在约0.3到约0.8mg/kg之间并且每2周施用一次。在某些实施方式中，所述初始剂量是约0.1mg/kg、约0.13mg/kg、约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg，并且每1、2、3、4、5或6周施用一次。。在某些实施方式中，所述初始剂量是约0.3mg/kg、约0.4mg/kg、约0.5mg/kg、约0.6mg/kg、约0.7mg/kg、约0.8mg/kg、约0.9mg/kg、约1.0mg/kg、约1.1mg/kg、约1.2mg/kg、约1.3mg/kg、约1.4mg/kg或约1.5mg/kg，并且每2周施用一次。。在某些实施方式中，所述初始剂量是约0.3mg/kg，并且每2周施用一次。在某些实施方式中，所述初始剂量是约0.5mg/kg，并且每2周施用一次。在某些实施方式中，所述初始剂量是约0.7mg/kg，并且每2周施用一次。在某些实施方式中，所述初始剂量是约0.8mg/kg，并且每2周施用一次。

[0392] 在某些实施方式中，所述剂量是调整的剂量。在某些实施方式中，所述调整的剂量大于所述初始剂量。在某些实施方式中，所述调整剂量是约2.5mg、约5mg、约10mg、约15mg、约20mg或约35mg大于所述初始剂量，或约0.05mg/kg、约0.1mg/kg、约0.15mg/kg、约0.25mg/kg、约0.3mg/kg、约0.35mg/kg、约0.4mg/kg或约0.5mg/kg大于所述初始剂量。在某些实施方式中，所述调整的剂量比所述初始剂量更频繁地施用。在某些实施方式中，所述调整的剂量每5、10、15、20、25、28、30、35或40天施用。

[0393] 在某些实施方式中，所述调整的剂量小于所述初始剂量。在某些实施方式中，所述调整的剂量是约2.5mg、约5mg、约10mg、约15mg、约20mg或约35mg小于所述初始剂量，或约0.05mg/kg、约0.1mg/kg、约0.15mg/kg、约0.25mg/kg、约0.3mg/kg、约0.35mg/kg、约0.4mg/kg或约0.5mg/kg小于所述初始剂量。在某些实施方式中，所述调整的剂量比所述初始剂量较少频繁地施用。在某些实施方式中，所述调整的剂量每30、35、40、42、50、60、70、80或90天施用。

[0394] 在某些实施方式中，所述剂量通过注射施用。在某些实施方式中，所述剂量每28天施用一次，或每42天施用一次。在某些实施方式中，连续地和/或无限地施用所述剂量。

[0395] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中激活素的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中激活素的水平和/或活性至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、

80%、85%、90%、95%或最多100%。在某些实施方式中，所述激活素是游离激活素，例如，不与卵泡抑素、卵泡抑素样3或抑制素缔合的激活素。在某些实施方式中，所述激活素是激活素A。在某些实施方式中，激活素水平和/或活性通过如章节8.6中描述的分析来测定。

[0396] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的Smad依赖性信号转导相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中的Smad依赖性信号转导至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，Smad依赖性信号转导通过如章节8.6中描述的分析来测定。

[0397] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中Runx2的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中Runx2的水平和/或活性至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，Runx2水平和/或活性通过如章节8.6中描述的分析来测定。

[0398] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中Alp的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中Alp的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，Alp水平和/或活性通过如章节8.6中描述的分析来测定。

[0399] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中Snail的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中Snail的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，Snail水平和/或活性通过如章节8.6中描述的分析来测定。

[0400] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中phosphosmad2的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中phosphosmad2的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，phosphosmad2水平和/或活性通过如章节8.6中描述的分析来测定。

[0401] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中

phosphosmad3的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中phosphosmad3的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，phosphosmad3水平和/或活性通过如章节8.6中描述的分析来测定。

[0402] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中尿蛋白的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中尿蛋白的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，尿蛋白水平和/或活性通过如章节8.6中描述的分析来测定。

[0403] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中Dkk1的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中Dkk1的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，Dkk1水平和/或活性通过如章节8.6中描述的分析来测定。

[0404] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中colla1的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中colla1的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，colla1水平和/或活性通过如章节8.6中描述的分析来测定。

[0405] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中BSAP的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中BSAP的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，BSAP水平和/或活性通过如章节8.6中描述的分析来测定。

[0406] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中CTX的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中CTX的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、

85%、90%、95%或最多100%。在某些实施方式中，CTX水平和/或活性通过如章节8.6中描述的分析来测定。

[0407] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中Osterix的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中Osterix的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，Osterix水平和/或活性通过如章节8.6中描述的分析来测定。

[0408] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中ActRIIA的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中ActRIIA的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，ActRIIA水平和/或活性通过如章节8.6中描述的分析来测定。

[0409] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中Klotho的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象中Klotho的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些实施方式中，Klotho水平和/或活性通过如章节8.6中描述的分析来测定。

[0410] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中alpha-SMA的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象中alpha-SMA的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些实施方式中，alpha-SMA水平和/或活性通过如章节8.6中描述的分析来测定。

[0411] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中MYOCD的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象中MYOCD的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些

实施方式中，MYOCD水平和/或活性通过如章节8.6中描述的分析来测定。

[0412] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中Axin2的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象中Axin2的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些实施方式中，Axin2水平和/或活性通过如章节8.6中描述的分析来测定。

[0413] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中血管平滑肌蛋白质水平相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象中血管平滑肌蛋白质水平，例如，Sm22-alpha。在某些实施方式中，血管平滑肌蛋白质水平和/或活性通过如章节8.6中描述的分析来测定。在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中Sm22-alpha的水平和/或活性相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象中Sm22-alpha的水平和/或活性达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些实施方式中，Sm22-alpha水平和/或活性通过如章节8.6中描述的分析来测定。

[0414] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的骨体积相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象中的骨体积达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，骨体积通过如章节8.6中描述的分析来测定。

[0415] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中破骨细胞凹陷表面相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中破骨细胞凹陷表面达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，破骨细胞凹陷表面通过如章节8.6中描述的分析来测定。

[0416] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的骨形成率相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以维持所述对象的骨形成率，或最低限度地提高或降低所述对象的骨形成率，例如，最多1%、2.5%、5%、10%或15%。在某些实施方式中，骨形成率通过如章节8.6中描述的分析来测定。

[0417] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的成骨细

胞表面相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以维持所述对象的成骨细胞表面，或最低限度地提高或降低所述对象的成骨细胞表面，例如，最多1％、2.5％、5％、10％或15。在某些实施方式中，成骨细胞表面通过如章节8.6中描述的分析来测定。

[0418] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中主动脉的成骨细胞转变相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中主动脉的成骨细胞转变达至少5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或至少100％，或最多5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或最多100％。在某些实施方式中，主动脉的成骨细胞转变通过如章节8.6中描述的分析来测定。

[0419] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的成骨细胞数量相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的成骨细胞数量达至少5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或至少100％，或最多5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或最多100％。在某些实施方式中，成骨细胞数量通过如章节8.6中描述的分析来测定。

[0420] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的成骨细胞表面与骨表面比相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的成骨细胞表面与骨表面比达至少5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或至少100％，或最多5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或最多100％。在某些实施方式中，成骨细胞表面与骨表面比通过如章节8.6中描述的分析来测定。

[0421] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的破骨细胞数量相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的破骨细胞数量达至少5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或至少100％，或最多5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或最多100％。在某些实施方式中，破骨细胞数量通过如章节8.6中描述的分析来测定。

[0422] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的破骨细胞表面与骨表面比相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的破骨细胞表面与骨表面比达至少5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或至少100％，或最多5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或最多100％。在某些实施方式中，破骨细胞表面与骨表面比通过如章节8.6中描述的分析来测定。

[0423] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的小梁骨体积相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述

对象的小梁骨体积达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些实施方式中，小梁骨体积通过如章节8.6中描述的分析来测定。

[0424] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的小梁厚度相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象的小梁厚度达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些实施方式中，小梁厚度通过如章节8.6中描述的分析来测定。

[0425] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的血管平滑肌功能相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象的血管平滑肌功能达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些实施方式中，血管平滑肌功能通过如章节8.6中描述的分析来测定。

[0426] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的血管钙化相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的血管钙化达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，血管钙化通过如章节8.6中描述的分析来测定。在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的血管钙水平相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的血管钙水平达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，血管钙水平通过如章节8.6中描述的分析来测定。

[0427] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的主动脉钙水平相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的主动脉钙水平达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、

95%或最多100%。在某些实施方式中，主动脉钙水平通过如章节8.6中描述的分析来测定。在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)的主动脉粥样斑中的钙沉积相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的主动脉粥样斑中的钙沉积达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，主动脉粥样斑中的钙沉积通过如章节8.6中描述的分析来测定。

[0428] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的EnMT相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象中CKD诱导的内皮向间充质转变(EnMT)达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，EnMT通过如章节8.6中描述的分析来测定。

[0429] 在某些实施方式中，与参考群体(例如如章节8.6中描述的参考群体)中的心脏大小(例如，心脏重量)相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的心脏大小(例如，心脏重量)达至少1%、2%、3%、4%、5%、6%、7%、8%、9%或至少10%，或最多1%、2%、3%、4%、5%、6%、7%、8%、9%或至少10%。在某些实施方式中，心脏大小通过如章节8.6中描述的分析来测定。

[0430] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的分化的血管平滑肌细胞水平相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高所述对象的分化的血管平滑肌细胞水平达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或至少500%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%、100%、125%、150%、175%、200%、250%、300%、350%、400%、450%或最多500%。在某些实施方式中，血分化的血管平滑肌细胞水平通过如章节8.6中描述的分析来测定。

[0431] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的动脉硬化相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的动脉硬化水平升高达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，动脉硬化通过如章节8.6中描述的分析来测定。在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的动脉硬化相比，根据本文提供的方法施用的ActRII信号转导抑制剂的剂量足以降低所述对象的动脉硬化水平升高达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、

35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，动脉硬化通过如章节8.6中描述的分析来测定。

[0432] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的矿物质合并率相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以维持所述对象的矿物质合并率，或最低限度地提高或降低所述对象的最小合并率，例如，最多1%、2.5%、5%、10%或15%。在某些实施方式中，矿物质合并率通过如章节8.6中描述的分析来测定。

[0433] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的高磷酸血症相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以维持所述对象的高磷酸血症，或最低限度地提高或降低所述对象的高磷酸血症，例如，最多1%、2.5%、5%、10%或15%。在某些实施方式中，高磷酸血症通过如章节8.6中描述的分析来测定。

[0434] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的FGF23水平相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以维持所述对象的FGF23水平，或最低限度地提高或降低所述对象的FGF23水平，例如，最多1%、2.5%、5%、10%或15%。在某些实施方式中，FGF水平和/或活性通过如章节8.6中描述的分析来测定。

[0435] 在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的肾脏纤维化相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的肾脏纤维化达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，肾脏纤维化通过如章节8.6中描述的分析来测定。在某些实施方式中，与参考群体(例如，如章节8.6中描述的参考群体)中的肾小球硬化症相比，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以降低所述对象的肾小球硬化症达至少5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或至少100%，或最多5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或最多100%。在某些实施方式中，肾小球硬化症通过如章节8.6中描述的分析来测定。

[0436] 在某些实施方式中，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以归一化本文提供的一种或更多种生物标记物(例如，Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、尿蛋白和/或ActRIIA)的水平。例如，在某些实施方式中，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以提高或降低本文提供的一种或更多种生物标记物(例如，Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、尿蛋白和/或ActRIIA)的水平到参考群体(例如，如章节8.6中描述的参考群体)中相应生物标记物的水平。

[0437] 在某些实施方式中，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的

剂量足以治疗和/或预防所述对象的心脏肥大。在某些实施方式中，心脏肥大通过如章节8.6中描述的分析来测定。在某些实施方式中，根据本文提供的方法向对象施用的ActRII信号转导抑制剂的剂量足以治疗和/或预防所述对象的LVH。

[0438] 当与本文提供的剂量(例如，ActRII信号转导抑制剂的剂量或第二活性试剂的剂量)一起使用时，词语“约”是指参考数量的1％、5％或10％以内的任何数量。

[0439] 在某些实施方式中，本文描述的ActRII信号转导抑制剂根据本文提供的方法皮下地或静脉内地施用给对象。

[0440] 在某些实施方式中，0.13mg/kg的ActRIIA-hFc(SEQ ID NO:7)以每14天一次的间隔皮下地施用给根据所提供的方法治疗的对象。在某些实施方式中，0.26mg/kg的ActRIIA-hFc(SEQ ID NO:7)以每14天一次的间隔皮下地施用给根据所提供的方法治疗的对象。在某些实施方式中，0.1mg/kg的ActRIIA-hFc(SEQ ID NO:7)以每14天一次的间隔静脉内地施用给根据所提供的方法治疗的对象。在某些实施方式中，0.2mg/kg的ActRIIA-hFc(SEQ ID NO:7)以每14天一次的间隔静脉内地施用给根据所提供的方法治疗的对象。在某些实施方式中，0.3mg/kg的ActRIIA-hFc(SEQ ID NO:7)以每28天一次的间隔皮下地施用给根据所提供的方法治疗的对象。在某些实施方式中，0.5mg/kg的ActRIIA-hFc(SEQ ID NO:7)以每28天一次的间隔皮下地施用给根据所提供的方法治疗的对象。在某些实施方式中，0.7mg/kg的ActRIIA-hFc(SEQ ID NO:7)以每28天一次的间隔皮下地施用给根据所提供的方法治疗的对象。

[0441] 8.8 组合治疗

[0442] 在某些实施方式中，本文提供的方法与第二药学活性试剂组合地进行。这样的组合治疗可以通过治疗的单独成分的同时、连续或独立给药的方式来实现。此外，当作为这种组合治疗的成分施用时，ActRII信号转导抑制剂与第二药学活性试剂可以是协同的，从而与通常作为单一治疗给予的每种成分的剂量相比，两种成分的任一种或两种的日剂量可以被降低。做为选择，当作为这种组合治疗的成分施用时，本文提供的ActRII信号转导抑制剂与第二药学活性试剂可以是加成的，从而与通常作为单一治疗给予的每种成分的剂量相比，每种成分的日剂量是相似的或相同的。

[0443] 在某些实施方式中，本文提供的ActRII信号转导抑制剂与第二药学活性试剂在同一天施用。在某些实施方式中，ActRII信号转导抑制剂在第二药学活性试剂之前一天、两天、三天或更多天施用。在某些实施方式中，ActRII信号转导抑制剂在第二药学活性试剂之后一天、两天、三天或更多天施用。在某些实施方式中，ActRII信号转导抑制剂在第二药学活性试剂一周、两周、三周或更多周之内施用。

[0444] 在某些实施方式中所述第二药学活性试剂是Snail、phosphosmad2、phosphosmad3、尿蛋白、Dkk1、colla1、激活素(例如，游离激活素)、Runx2、Alp、BSAP、CTX和/或Osterix的拮抗剂，例如抗体或其片段、小分子信号转导抑制剂、反义核酸、小干扰核酸、显性阴性蛋白或其片段。在某些实施方式中，所述第二药学活性试剂是Klotho、alpha-SMA、MYOCD、Axin2和/或Sm22-alpha的激动剂，例如，抗体或其片段或小分子

[0445] 在某些实施方式中，所述第二药学活性试剂是用于治疗心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病和/或与肾病相关和/或由肾病引起的心血管疾病的活性试剂，例如，醛固酮信号转导抑制剂、血管紧张素II受体阻断剂、beta-阻断

剂、钙离子通道阻断剂、降胆固醇药物、地高辛、利尿剂、变力性治疗、钾或镁、血管扩张剂和/或华法林。

[0446] 在某些实施方式中，所述第二药学活性试剂是用于治疗慢性肾病的活性试剂，例如，血管紧张素II受体阻断剂、beta-阻断剂、钙离子通道阻断剂、直接肾素信号传导抑制剂、利尿剂、血管舒张剂、促红细胞生成素治疗、铁替代治疗和/或维生素D.

[0447] 在某些实施方式中，与治疗或改善心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病和/或与肾病相关和/或由肾病引起的心血管疾病、和/或慢性肾病的方法组合地进行本文提供的方法。

[0448] 8.9 药物组合物

[0449] 在某些实施方式中，激活素-ActRII拮抗剂(例如，ActRII多肽)与药学上可接受的载体一起配制用于本文描述的方法。例如，ActRII多肽可以单独施用或作为药物制剂的成分(治疗组合物)施用。目标化合物可以被配制以用于人类或兽医学的任何方便方式施用。ActRII可以是ActRIIA或ActRIIB。

[0450] 在某些实施方式中，本文提供的治疗方法包括系统地或作为植入物或设备局部地施用所述组合物(包含ActRII信号转导抑制剂)。当施用时，用于本文提供的用途的治疗组合物是无热原的、生理学可接受的形式。还可以任选地被包括在如上所述的组合物中的ActRII拮抗剂之外的治疗有用试剂，可以与目标化合物(例如，ActRII多肽，例如，ActRIIA和/或ActRIIB多肽(参见，章节8.5))同时地或次序地施用。

[0451] 一般地，ActRII拮抗剂将胃肠外施用。适合于胃肠外施用的药物组合物可以包含一种或更多种ActRII多肽，与一种或更多种药学上可接受的无菌的等渗水性或非水性溶液、分散体、悬浮液或乳剂或无菌粉末组合，所述无菌粉末在即将使用之前可重构为无菌可注射溶液或分散体，其可以含有抗氧化剂、缓冲剂、抑菌剂、使得制剂与预期的接受者的血液等渗的溶质，或悬浮剂或增稠剂。在用于本文描述的方法中的药物组合物中采用的适合的水性和非水性载体的实例包括，水、乙醇、多元醇(例如，甘油、丙二醇、聚乙二醇，等等)、以及其适合的混合物，植物油，例如橄榄油，和可注射的有机酯，例如油酸乙酯。例如，通过使用包被材料例如卵磷脂、在分散体的情况下通过维持所需的粒子大小、以及通过使用表面活性剂，可以维持适当的流动性。

[0452] 进一步的，所述组合物可以以用于递送到目标组织位点(例如，骨)的形式被胶囊化或注射。在某些实施方式中，本文描述的方法中使用的组合物可以包括基质，其能将一种或更多种治疗化合物(例如，ActRIIA多肽)递送到目标组织位点(例如，骨)，提供一种结构用于显现组织以及最佳地能够被再吸收入身体。例如，所述基质可以提供ActRIIA多肽的缓慢释放。这样的基质可以由当前用于其他植入医学应用的材料形成。

[0453] 基质材料的选择基于生物相容性、生物降解能力、机械性质、美容的外观和接触面性质。目标组合物的特殊的应用将限定适当的制剂。组合物的可能的基质可以是生物可降解的和化学上定义的硫酸钙、磷酸三钙、羟基磷灰石、聚乳酸和聚酐。其他可能的材料是生物可降解的和生物学上明确定义的，例如骨或真皮胶原蛋白。进一步的基质包含纯的蛋白质或细胞外基质成分。其他可能的基质是非生物可降解的和化学上定义的，例如，烧结的羟磷灰石、生物玻璃、铝酸盐或其他陶瓷。基质可以包含任何上述材料类型的组合，例如聚乳酸和羟磷灰石，或胶原蛋白和磷酸三钙。生物陶瓷可以在组成上改变，例如在钙-铝酸盐-磷

酸盐中，并被加工来改变孔径大小、颗粒大小、颗粒形状和生物降解能力。

[0454] 在某些实施方式中，用于本文描述的方法的组合物(包含ActRII信号转导抑制剂)可以口服施用，例如以胶囊、扁胶囊、丸剂、片剂、锭剂(使用调味的基底，通常是蔗糖和阿拉伯胶或黄芪胶)、粉末、颗粒或作为水性或非水性液体中的溶液或悬浮液，或作为水包油或油包水液体乳剂或作为酏剂或糖浆或作为软锭剂(使用惰性基底，例如明胶和甘油，或蔗糖和阿拉伯胶)和/或作为漱口剂等的形式，各自含有预定数量的试剂作为活性成分。试剂也可以作为弹丸、舔剂或糊剂来施用。

[0455] 在用于口服施用的固体剂型中(胶囊、片剂、药丸、糖衣丸、粉末、颗粒，等)，本文描述的一种或更多种治疗化合物可以与一种或多种药学上可接受的载体，例如柠檬酸钠或磷酸二钙和/或任何以下的混合：(1)填料或膨胀剂，例如淀粉、乳糖、蔗糖、葡萄糖、甘露醇和/或硅酸；(3)粘合剂，例如羧甲基纤维素、藻酸盐、明胶、聚乙烯基吡咯烷酮、蔗糖和/或阿拉伯树胶；(3)湿润剂，例如甘油；(4)崩解剂，例如，琼脂、碳酸钙、马铃薯或木薯淀粉、海藻酸、某些硅酸盐和碳酸钠；(5)溶液阻滞剂，例如，石蜡；(6)吸收加速剂，例如，季铵化合物；(7)润湿剂，例如，十六醇和单硬脂酸甘油酯；(8)吸附剂，例如，高岭土和班脱土；(9)润滑剂，例如，滑石粉、硬脂酸钙、硬脂酸镁、固体聚乙二醇、十二烷基硫酸钠和其混合物；和(在胶囊、片剂和丸剂的情况中，药物组合物还可以包含缓冲剂。类似类型的固体组合物也可以用作软和硬填充胶囊中的填料，使用这样的赋形剂如乳糖或奶糖，以及高分子量聚乙二醇等等。

[0456] 用于口服施用的液体剂型包括药学上可接受的乳剂、微乳剂、溶液、悬浮液、糖浆和酏剂。除了活性成分之外，液体剂型可以含有本领域常用的惰性稀释剂，例如，水或其他溶剂、增溶剂以及乳化剂，例如乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苯甲醇、苯甲酸苄酯、丙二醇、1,3-丁二醇、油(特别是，棉籽、花生、玉米、胚芽、橄榄、蓖麻以及芝麻油)、甘油、四氢呋喃醇类、聚乙二醇以及山梨聚醇的脂肪酸酯，和其混合物。除了惰性稀释剂之外，口腔组合物还可以包括佐剂例如润湿剂、乳化和悬浮剂、甜味剂、调味剂、着色剂、芳香剂和防腐剂。

[0457] 除了活性化合物之外，悬浮液可以含有悬浮剂，例如，乙氧基异十八烷醇、聚氧乙烯山梨醇和山梨聚糖酯，微晶纤维素、偏氢氧化铝、斑脱土、琼脂和黄芪胶，以及其混合物。

[0458] 本文描述的组合物还可以含有佐剂，例如，防腐剂、润湿剂、乳化剂和分散剂。可以通过包含各种抗菌和抗真菌剂，例如，对羟苯甲酸、氯代丁醇、苯酚、山梨酸，等，来实现对微生物作用的保护。还可能希望的是在组合物中包括等渗试剂，例如糖类、氯化钠等。此外，通过包含延迟吸收的试剂，例如，单硬脂酸铝和明胶，可以得到可注射药物形式的延长吸收。

[0459] 要理解的是，给药方案将由主治医师考虑修饰本文描述的化合物(例如，ActRII多肽，例如，ActRIIA和/或ActRIIB多肽)的作用的各种因素来决定。所述各种因素包括但不限于，希望形成的骨重量的数量，骨密度损失程度，骨损伤部位，骨损伤状况，对象的年龄、性别和饮食，可能导致骨损失的任何疾病的严重度，施用的时间和其他临床因素。任选地，所述剂量可以随重构中使用的基质的类型以及组合物中化合物的类型而变化。其他已知的生长因子向最终组合物中的添加也可能影响剂量。进展可以通过周期性评估骨生长和/或修复来监视，例如，X-射线(包括DEXA)、组织形态学测定和四环素标记。

[0460] 在某些实施方式中，本文提供的是用于ActRII多肽的体内生产的基因治疗。通过将ActRII多核苷酸序列导入具有上文列出的失调的细胞或组织，这样的疗法将实现其治疗

效果。递送ActRII多核苷酸序列可以利用重组表达载体，例如嵌合病毒或胶态分散体系统来实现。优选的，用于ActRII多核苷酸序列的治疗递送的是使用靶向的脂质体。ActRII多肽可以是ActRIIA和/或ActRIIB多肽(参见，章节8.5))。

[0461] 在此教导的可以用于基因治疗的各种病毒载体包括腺病毒、疱疹病毒、牛痘，或优选地，RNA病毒，例如逆转录病毒。优选地，逆转录病毒载体是鼠或禽类逆转录病毒的衍生物。其中可以插入单个外源基因的逆转录病毒载体的实例包括，但不限于，Moloney鼠白血病毒(MoMuLV)、Harvey鼠肉瘤病毒(HaMuSV)、鼠类乳腺肿瘤病毒(MuMTV)和劳氏肉瘤病毒(RSV)。许多其他逆转录病毒载体可以掺入多个基因。所有这些载体可以传递或掺入可选择标记物的基因，从而可以鉴定和产生转导的细胞。通过附着例如糖类、糖脂或蛋白质，逆转录病毒载体可以变为目标特异性的。优选的靶向是通过使用抗体实现的。技术人员将认识到，特异性多核苷酸序列可以插入逆转录病毒基因组中或附着于病毒包膜，以容许含有ActRII多核苷酸的逆转录病毒载体的目标特异性递送。在优选的实施方式中，所述载体被靶向骨或软骨。

[0462] 做为选择，通过常规的磷酸钙转染，组织培养细胞可以直接用编码逆转录病毒结构基因gag、pol和env的质粒转染。这些细胞然后用含有感兴趣基因的载体质粒转染。产生的细胞将逆转录病毒载体释放到培养基中。

[0463] ActRII多核苷酸的另一种靶向递送系统是胶态分散体系统。胶态分散体系统包括大分子复合物、纳囊、微球体、珠子和基于脂质的系统，包括水包油乳剂、胶粒团、混合的胶粒团和脂质体。用于本文描述的方法的优选的胶态系统是脂质体。脂质体是人工的膜囊，其作为体外和体内的递送运载体是有用的。RNA、DNA和完整的病毒粒可以密封在水性的内部，并以生物学活性形式递送给细胞(参见，例如，Fraley,et al.,Trends Biochem.Sci.,6:77,1981)。利用脂质体载体的高效的基因转移方法是本领域已知的，参见，例如，Mannino,et al.,Biotechniques,6:682,1988。脂质体的组成通常是磷脂类的组合，通常与类固醇，特别是胆固醇组合。也可以使用其他磷脂或其他脂质。脂质体的物理性质取决于pH值、离子强度和二价阳离子的存在。

[0464] 在脂质体生产中有用的脂质包括磷脂酰基化合物，例如，磷脂酰甘油、磷脂酰胆碱、磷脂酰丝氨酸、磷脂酰乙醇胺、鞘脂类、脑苷脂类和神经节苷脂类。说明性的磷脂包括卵磷脂酰胆碱、二棕榈酰磷脂酰胆碱和二硬脂酰磷脂酰胆碱。基于例如器官特异性、细胞特异性和细胞器特异性，脂质体的靶向也是可能的，是本领域已知的。

[0465] 在某些实施方式中，ActRII信号转导抑制剂在药物组合物中是基本上纯的。具体地，所述药物组合物中的最多20％、10％、5％、2.5％、1％、0.1％或最多0.05％的化合物是除了ActRII信号转导抑制剂和药学上可接受的载体之外的化合物。

[0466] 8.10 试剂盒

[0467] 本文提供的是包含一个或更多个容器的试剂盒，所述容器填充了一种或更多种试剂，来测定获自本文描述的对象的样品中本文描述的一种或更多种生物标记物(例如Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中，所述试剂盒包含一种或更多种试剂，来测定获自本文描述的对象的样品中本文描述的一种生物标记物(例如Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、

BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中Runx2的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中Alp的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中Snail的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中phosphosmad2的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中Dkk1的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中coll a1的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中激活素的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中BSAP的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中CTX的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中osterix的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中Klotho的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中alpha-SMA的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中MYOCD的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中Sm22-alpha的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中phosphosmad3的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中尿蛋白的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中ActRIIA的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中Axin2的水平。

[0468] 在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的两种生物标记物(例如选自以下构成的组的两种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、coll a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的三种生物标记物(例如选自以下构成的组的三种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、coll a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad3、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的四种生物标记物(例如选自以下构成的组的四种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、coll a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad四、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的五种生物标记物(例如选自以下构成的组的五种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、coll a1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad五个、ActRIIA、Axin2和

尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的六种生物标记物(例如选自以下构成的组的六种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad六、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的七种生物标记物(例如选自以下构成的组的七种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad七、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的八种生物标记物(例如选自以下构成的组的八种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad八、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的九种生物标记物(例如选自以下构成的组的九种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad九、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的十种生物标记物(例如选自以下构成的组的十种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的十一种生物标记物(例如选自以下构成的组的十一种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的十二种生物标记物(例如选自以下构成的组的十二种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的十三种生物标记物(例如选自以下构成的组的十三种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的十四种生物标记物(例如选自以下构成的组的十四种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的十五种生物标记物(例如选自以下构成的组的十五种生物标记物:Runx2、Alp、Snail、phosphosmad2、Dkk1、colla1、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述

的对象的样品中本文描述的十六种生物标记物(例如选自以下构成的组的十六种生物标记物:Runx2、Alp、Snai1、phosphosmad2、Dkk1、collal、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的十七种生物标记物(例如选自以下构成的组的十七种生物标记物:Runx2、Alp、Snai1、phosphosmad2、Dkk1、collal、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述试剂盒包含一种或更多种试剂,来测定获自本文描述的对象的样品中本文描述的十八种生物标记物(例如选自以下构成的组的十八种生物标记物:Runx2、Alp、Snai1、phosphosmad2、Dkk1、collal、激活素、BSAP、CTX、osterix、Klotho、alpha-SMA、MYOCD、Sm22-alpha、phosphosmad十、ActRIIA、Axin2和尿蛋白)的水平。在某些实施方式中,所述测定生物标记物的水平的一种或更多种试剂是如章节8.6.1中描述的。在某些实施方式中,所述试剂盒进一步包含ActRIIA-hFc(SEQ ID NO:7)。在某些实施方式中,所述试剂盒进一步包含第二容器,其包含ActRIIA-hFc(SEQ ID NO:7)。

9.实施例

[0469] 在此呈现的实施例表明,在某些形式的慢性肾病中Runx2、Alp、BSAP、CTX和Osterix mRNA水平升高,Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和Sm22-alpha mRNA水平可降低。这些实施例进一步表明,用激活素配体陷阱治疗降低了Runx2、Alp、CTX、ActRIIA和/或Osterix mRNA水平,提高了Klotho、alpha-SMA、MYOCD、Axin2和/或Sm22-alpha mRNA水平,这与血管钙化的降低相关。因而,本文提供的实施例表明Runx2、Alp、CTX、Osterix、Klotho、alpha-SMA、MYOCD、ActRIIA、Axin2和/或Sm22-alpha可以用作生物标记物,用于治疗心血管疾病、血管钙化、与血管钙化相关和/或由血管钙化引起的心血管疾病、与肾病相关和/或由肾病引起的心血管疾病、动脉硬化水平升高、与动脉硬化水平升高相关和/或由动脉硬化水平升高引起的心血管疾病、LVH和/或与LVH相关和/或由LVH引起的心血管疾病。

[0470] 9.1 实施例1.用激活素受体2A型的配体陷阱治疗CKD-MBD

[0471] CKD-MBD可以包括血管钙化,一种骨营养不良,以及在它发端时对骨骼的骨细胞FGF23分泌的刺激,以及高磷酸血症,其在进程的晚期发生进一步刺激血管钙化。这个实施例表明,抑制ActRIIA信号转导,一种由CKD诱导的、通过激活素IIA型受体(ActRIIA)的TGFβ超家族信号转导的成员,抑制了血管钙化并预防心脏肥大。

[0472] 9.1.1 方法

[0473] 带有高磷酸血症和GFR 60%降低的CKD(CKD-3)在2型糖尿病ldlr-/-小鼠、血管钙化高脂肪饲喂模型中在14周龄诱导。一些CKD小鼠用激活素2A型受体(mActRIIA)配体陷阱mActRIIA-Fc治疗,在22周龄开始每周IP注射,在28周时研究。测量了主动脉的Ca^{2+}水平、成骨细胞的和血管平滑肌蛋白质的表达、骨骼组织形态和microCT成像、血清化学以及FGF23和PTH水平。激活素、卵泡抑素和抑制素水平通过ELISA、RT-PCR和Western印迹测量。

[0474] 9.1.2 结果

[0475] 循环激活素水平在两种不同的肾脏疾病模型中提高:间质性纤维化和X-连锁AIports(附图1A和附图1B)。此外,激活素A mRNA水平在CKD模型的主动脉和肾脏中提高(附

图2)。激活素水平在血管系统和循环中提高,而没有卵泡抑素的改变(附图2A、附图2B和附图2C)。通过用激活素2A型受体(mActRIIA)配体陷阱(mActRIIA-Fc;参见,例如美国专利8,173,601,其公开内容通过完全引用合并在本文中)治疗,CKD刺激的血管钙化降低到低于22周的水平(附图3A、附图3B、附图3C、附图3D、附图5A和附图5B),预防了心脏肥大。CKD诱导了主动脉的Runx2、osterix和Alp信息和蛋白质的表达,这些被激活素2A型受体(mActRIIA)配体陷阱mActRIIA-Fc反转(对于Runx2和Alp mRNA,分别是附图4A和附图4B;对于Runx2蛋白,附图4F)。CKD降低了Klotho和Sm22-alpha信息和蛋白质,用激活素2A型受体(mActRIIA)配体陷阱mActRIIA-Fc治疗归一化了Klotho和Sm22-alpha表达(对Klotho和Sm22-alpha mRNA分别是附图4C和附图4E;附图4F为蛋白质)。此外,CKD降低了MYOCD信息(附图4D)。此外,CKD降低了alpha-平滑肌肌动蛋白(肌动蛋白alpha-平滑肌)蛋白质(附图4F)。进一步的,CKD刺激了钙水平的提高,其被mActRIIA-Fc治疗降低(附图6A)。激活素2A型受体(mActRIIA)配体陷阱mActRIIA-Fc治疗提高了骨体积,破骨细胞凹陷表面降低,但是骨形成率和成骨细胞表面不受影响(附图6B、附图6C和附图6D)。激活素2A型受体(mActRIIA)配体陷阱mActRIIA-Fc治疗没有改变高磷酸血症和FGF23水平。

[0476] 用激活素2A型受体(mActRIIA)配体陷阱mActRIIA-Fc治疗抑制了骨吸收并提高了骨体积。用激活素2A型受体(mActRIIA)配体陷阱mActRIIA-Fc治疗抑制了Smad依赖性信号转导,阻断了主动脉的成骨细胞的转变,提高了血管平滑肌蛋白质水平,并降低了CKD刺激的血管钙化和心脏肥大。

[0477] 9.2 实施例2.慢性肾病(CKD)刺激主动脉的内皮向间充质转变(ENMT),其引起血管钙化并被激活素配体陷阱抑制

[0478] 在慢性肾病中血管钙化的基础分子机制是不完全了解的。然而,激活素水平在CKD刺激的血管钙化中提高。为了评估激活素在CKD刺激的血管钙化中的作用,结合许多TGF-beta超家族配体的重组融合蛋白被用于动脉粥样硬化和2型糖尿病的模型中的慢性肾病的小鼠模型中。ActRIIA-Fc融合蛋白由同免疫球蛋白1(IgG1)Fc结构域连接的激活素受体IIA(ActRIIA)的细胞外结构域组成,该蛋白质作为TGF-beta超家族成员如激活素A、激活素B、生长分化因子-11(GDF11)和骨骼形态发生蛋白-10(BMP-10)的配体陷阱起作用。

[0479] 早先已经显示了,通过在肾脏修复期间产生全身性Wnt抑制,肾脏疾病引起血管钙化。这个实施例表明,CKD刺激的Wnt抑制的血管作用是刺激血管的激活素,以及诱导Smad依赖性EnMT,激活素受体配体陷阱抑制了EnMT和血管钙化。

[0480] 9.2.1 方法

[0481] 具有升高的Wnt抑制剂的CKD在谱系跟踪和血管钙化的小鼠模型中诱导。激活素、卵泡抑素和抑制素水平通过ELISA、RT-PCR和Western印迹测量。在繁育成内皮特异性Tie2-Cre小鼠的GNZ小鼠(Stoller et al,Genesis,2008)中进行细胞谱系跟踪。带有GNZ敲入的小鼠在Cre介导的重组之后表达核GFP和lacZ。

[0482] 9.2.2 结果

[0483] 在肾脏疾病和CKD刺激的血管钙化的小鼠模型中,激活素水平在血管系统和循环中提高,而没有卵泡抑素水平的改变。在GNZ的外膜和中层的细胞中CKD诱导GFP和lacZ的表达;Tie2-Cre CKD小鼠与GNZ相比较;Tie2-Cre小鼠具有正常的肾脏功能,其中GFP和lacZ限于主动脉的内皮。Tie2是内皮的谱系特异性受体,这表明CKD诱导主动脉的EnMT(附图7A、附

图7B、附图7C和附图7D)。CKD刺激的主动脉EnMT产生了降低的血管平滑肌功能,成骨细胞转变和钙化。用激活素2A型受体(mActRIIA)配体陷阱(mActRIIA-Fc;参见,例如,美国专利No.8,173,601,其公开内容通过引用全部合并在本文中)治疗抑制了Smad依赖性信号转导,阻断了主动脉的成骨细胞转变,提高了血管平滑肌功能,并降低了CKD刺激的血管钙化。因而,CKD诱导血管激活素和EnMT,用激活素2A型受体(mActRIIA)配体陷阱治疗降低了激活素信号转导抑制的血管去分化,成骨细胞转变和血管钙化。

[0484] 9.3 实施例3.以逐步提高的剂量治疗的血液透析对象中骨质量和血管钙化的初步信号搜寻定量计算机断层扫描结果

[0485] 高周转的肾性骨营养不良(ROD)的标志是提高的皮层孔隙度、更高的小梁骨质量和提高的骨折风险,一种在矫正血液透析(HD)对象的贫血中研究的激活素ARIIA-IgG1融合蛋白配体陷阱ActRIIA-Fc阻断了激活素A信号转导,可以降低破骨细胞发生并促进骨中的成骨细胞成熟。利用定量的计算机断层照相(QCT),在HD对象中的当前的分析评估了ActRIIA-Fc对骨矿物密度(BMD)和血管钙化的影响。

[0486] 9.3.1 方法

[0487] 用ActRIIA信号转导抑制剂治疗的、促红细胞生成素刺激剂(ESA)响应性的血液透析对象被洗出ESA作用直到血红蛋白(Hb)<10g/dL,然后随机化为用以下剂量的ActRIIA信号转导抑制剂治疗:0.3mg/kg(n=9)、0.5mg/kg(n=8)、0.7mg/kg(n=6)或安慰剂(PBO;n=7)每28天皮下地,直到8个剂量周期。评估对象的Hb、骨矿物密度(BMD)和骨周转的生物标记物的影响。治疗失败(Hb<9g/dL)用ESA和/或红血细胞输血来挽救。在基线和225天的治疗期之后获得髋部和腰脊椎的定量计算机断层扫描(QCT)。在基线和剂量周期3、5和7之后测量生物标记物BSAP和CTX。

[0488] 在基线和225天的治疗期之后获得髋部、腰脊椎和腹主动脉的QCT。对象仰卧在Mindways校准模型上(Model 3;Mindways Software,Inc.,Austin,TX)。使用标准软组织核重建2.5mm和512×512矩阵的切片厚度。Mindways分析软件(版本5.0.3)用于评估体积的BMD(vBMD)。小梁的vBMD(mg/cm3)对L1-4内的2节脊椎测定(一般L1-2)。分析左侧股骨近端股骨头皮层,骨小梁和整个髋部的整合骨区室、股骨颈和髁枢的vBMD。

[0489] 使用软件来评估腹主动脉的血管钙化,所述软件半自动地分段邻近于L1顶部到L4底部的区域内钙化的面积和体积。在每个对象的就诊之间保持了切片的数量和位置。如Agatston et al.,J Am Coll Cardiol.1990;15:827-832 and Hokanson et al.,AJR AM J Roentgenol.2004;182:1327-1332中描述的测定Agatston和平方根转化的体积得分。更低的总Agatston和平方根转化的总体积得分(mm3)表明更低水平的血管钙化(VC)。

[0490] 通过PAREXEL成像(PAREXEL International Corp.Waltham,MA)中央地进行所有的图像质量控制和盲分析。

[0491] 在基线和剂量周期1、3、5和7之后测量生物标记物,包括骨特异性碱性磷酸酶(BSAP)、前胶原蛋白1型N-末端原肽(P1NP)和C-末端1型胶原蛋白端肽(CTX)。

[0492] 9.3.2 结果

[0493] 总共31名对象随机化,接受超过1个剂量的研究药物。

[0494] 表2.随机化的对象和QCT分析子集合

[0495]

对象,n	安慰剂	ActRIIA-Fc		
		0.3 mg/kg	0.5 mg/kg	0.7 mg/kg
随机化的并接受≥1 剂的研究药物	8	9	8	6
在基线和 225 天的 QCT 测量	3	6	5	2

[0496] 对象分布如附图8中描述,包括在基线和225天有成对的QCT测量的对象。

[0497] 大多数对象在治疗失败需要挽救(一般由于Hb<9g/dL)之后停止研究治疗;没有对象因为有害事件(AE)停止治疗。

[0498] 具有成对的QCT测量的16名对象中,9人在治疗期内需要挽救疗法,9人在前3个剂量周期内需要挽救。

[0499] 在具有成对的QCT测量的对象中,基线的人口统计学和临床特征一般在处理组之间是相似的(表3);然而,在安慰剂组中有实质上更长的透析时间,这也是最年轻的组。在基线生物标记物和Agatston得分方面各组之间也存在差异(表4)。

[0500] 表3.具有成对的QCT测量的对象的基线人口统计学和临床特征

[0501]

		ActRIIA-Fc		
	安慰剂 n=3	0.3 mg/kg n=6	0.5 mg/kg n=5	0.7 mg/kg n=2
年龄，平均，岁	54.0	57.3	60.8	69.0
女性， n（%）	0（0.0）	4（66.7）	0（0.0）	1（50.0）
种族， n（%）				
白人	1（33.0）	3（50.0）	3（60.0）	1（50.0）
黑人	1（33.0）	3（50.0）	2（40.0）	1（50.0）
亚洲人	1（33.0）	0（0.0）	0（0.0）	0（0.0）
族群， n（%）				
拉丁裔	0（0.0）	2（33.0）	3（60.0）	1（50.0）
非拉丁裔	3（100.0）	4（66.7）	2（40.0）	1（50.0）
透析后体重，平均，kg	70.4	80.3	79.4	84.4
体质量指数，平均，kg/m^2	25.4	27.7	26.9	29.2
糖尿病， n（%）	2（66.7）	5（83.3）	5(100.0)	2（100.0）
甲状旁腺切除， n（%）	0（0.0）	0（0.0）	0（0.0）	0（0.0）
在透析时间，平均，月	101.2	43.3	22.4	11.3
非钙磷酸盐结合剂， n（%）*	3（100.0）	3（50.0）	3（60.0）	0（0.0）
钙基磷酸盐结合剂，n（%）*	1（33.3）	4（66.7）	4（80.0）	2（100.0）
拟钙剂,n（%）*	1（33.3）	2（33.3）	0（0.0）	0（0.0）
1,25-OH 维生素 D 类似物,n(%)	2（66.7）	5（83.3）	2（40.0）	2（100.0）

[0502] 表4.具有成对的QCT测量的对象的平均基线生物标记物、面积BMD和Agatston得分

[0503]

		ActRIIA-Fc		
	安慰剂 n=3	0.3 mg/kg n=6	0.5 mg/kg n=5	0.7 mg/kg n=2
基线整体 PTH, pg/mL	261.4	104.8	135.5	100.4
BSAP, μg/L	28.3	18.0	13.2	7.5
P1NP, ng/mL	623.7	376.2	468.4	308.0
CTX, pg/mL	3,417.7	2,062.5	2,266.8	1,377.0
全髋整体 BMD, mg/cm^3	279.4	306.2	268.9	285.4
股骨颈皮层 BMD, mg/cm^3	653.1	666.9	593.1	566.5
平均脊柱(L1, L2) BMD, mg/cm^3	123.1	125.6	149.1	150.6
VC 总计 Agatston 得分*	8,665.0	9,472.7	3,618.5	823.2

[0504] PTH=甲状旁腺激素。

[0505] *更低的Agatston得分表明更低水平的血管钙化。

[0506] 表5提供了髋部整体、股骨颈皮层、髋部皮层和腰椎骨面积BMD测量中距基线改变百分比。

[0507] 表5.髋部整体、股骨颈皮层、髋部皮层和腰椎骨面积BMD中的基线和距基线改变百分比

[0508]

		ActRIIA-Fc		
	安慰剂 n=3	0.3 mg/kg n=6	0.5 mg/kg n=5	0.7 mg/kg n=2
全髋整体				
基线 BMD, mg/cm^3	279.4	306.2	268.9	285.4
% 距基线 BMD 的改变 (n)	-0.2 (3)	2.5 (5)	4.0 (5)	-1.8 (1)
股骨颈皮层				
基线 BMD (mg/cm^3)	653.1	666.9	593.1	565.5
%距基线 BMD 的改变 (n)	-0.9 (3)	-1.4 (5)	1.6 (5)	3.0 (1)
全髋, 皮层				
基线 BMD (mg/cm^3)	708.6	668.4	647.3	635.6
%距基线 BMD 的改变 (n)	-0.1 (3)	-1.1 (5)	0. 5 (5)	2.7 (1)
平均腰椎 (L1, L2)				
基线 BMD (mg/cm^3)	123.1	125.6	149.1	150.6
%距基线 BMD 的改变 (n)	12.6 (3)	8.0 (6)	0.5 (5)	-2.7 (2)

[0509] 皮层骨的2%提高可能降低骨折风险。在具有股骨颈部皮层骨的大于或等于2%提

高的部分对象中，ActRIIA信号转导抑制剂治疗与剂量依赖性提高相关(表5和附图9A)。

[0510] 在高周转ROD中，相比一般群体，小梁骨质量提高(即，不良质量的骨)，通过腰脊椎BMD来测量，在ESKD中在椎骨骨折率上没有降低。用ACTRIIA治疗预防了腰脊椎中小梁骨质量的提高(表5和附图9B)。

[0511] 腹主动脉总计Agatston得分的距基线改变在表6中提供。在总计Agatston得分和平方根转化的总体积得分方面改变的类别显示在附图9和表7中。

[0512] 表6.腹主动脉总计Agatston得分和平方根转化的总体积得分方面的基线和距基线改变。

[0513]

		ActRIIA-Fc		
	安慰剂 n=3	0.3 mg/kg n=6	0.5 mg/kg n=5	0.7 mg/kg n=2
腹主动脉总计 Agatston 得分				
基线总计 Agatston 得分	8,665	9,473	3,619	823
距基线总计 Agatston 得分的改变	1,050.4	8,578.9	225.7	43.4
%距基线总计 Agatston 得分的改变	58.4	24.9	17.3	3.4
平方根转化的总体积得分				
基线总体积平方根，mm^3	52.0	46.2	33.1	16.9
距基线总体积平方根的改变，mm^3	4.5	11.1	1.2	0.4

[0514] *更低的总计Agatston和平方根转化的总体积得分表明更低水平的血管钙化。

[0515] 十一位对象具有在10到10,000之间分布的基线Agatston得分。五名对象可以被认为是基线处的逸出值(低逸出值:0.0、1.1和1.4 Agatston得分;高逸出值:21,033和44,356 Agatston得分)。因此，分析没有逸出值的Agatston得分(表7和附图10)。

[0516] 表7.排除5个逸出值在总计Agatston得分方面的基线和改变

[0517]

		ActRIIA-Fc		
	安慰剂 n=1	0.3 mg/kg n=4	0.5 mg/kg n=4	0.7 mg/kg n=2
新的基线总计 Agatston 得分，排除 5 个逸出值	4,960	3,120	4,523	823
距基线总计 Agatston 得分的改变，排除 5 个逸出值	2,711.3	287.7	282.0	43.4
%距基线改变，排除 5 个逸出值	54.7	9.0	5.0	3.4

[0518] *更低的总计Agatston和平方根转化的总体积得分表明更低水平的血管钙化。

[0519] 通过对ActRIIA信号转导抑制剂的暴露持续时间分析对象。通过暴露状态分析对象：接受达到3个剂量的ActRIIA-Fc对象，不考虑剂量水平(n＝6)，接受>3个剂量的

ActRIIA-Fc接受者，不考虑剂量水平(n＝7)。安慰剂基团(n＝3)不变。除了性别和Agatston得分之外，在接受≤3个剂量的ActRIIA-Fc的对象与接受>3个剂量的对象之间，关键的基线特征一般是相似的，在所有ActRIIA-Fc对象中对VC的暴露影响保持完整，排除了逸出值时也是一样(表10)。

[0520] 表8.对象的基线人口统计学和临床特征，根据ActRIIA-Fc暴露

[0521]

	ActRIIA-Fc	
	≤3 剂 n=6	>3 剂 n=7
年龄，平均，岁	59.0	61.7
女性，n（%）	4（66.7）	1（14.3）
种族，n（%）		
白人	3（50.0）	4（57.1）
黑人	3（50.0）	3（42.9）
亚洲人	0（0.0）	0（0.0）
体质量指数，平均，kg/m^2	25.7	29.3
糖尿病，n（%）	5（83.3）	7（100.0）
在透析的时间，月	31.3	24.0
钙基磷酸盐结合剂，n（%）	4（66.7）	6（85.7）
拟钙剂，n（%）	1（16.7）	1（14.3）
1,25-OH 维生素 D 类似物，n（%）	4（66.7）	5（71.4）
基线整体 PTH，平均，pg/mL	104.5	125.7
BSAP，平均，μg/L	19.8	10.0
P1NP，平均，ng/mL	432.2	374.6
CTX，平均，pg/mL	2,410.3	1,714.4
VC 总计 Agatston 得分，平均	8,350	3,782
体积平方根，mm^3	38.2	35.3

[0522] 表9.根据ActRIIA-Fc暴露，在总计Agatston得分和平方根转化的总体积得分方面的基线和距基线改变*

[0523]

	ActRIIA-Fc	
	≤3 剂 n=6	>3 剂 n=7
基线总计 Agatston 得分	8,350	3,782
距基线总计 Agatston 得分的改变	8,461.4	274.3
% 距基线总计 Agatston 得分的改变	33.7	5.8
基线总体积平方根，mm^3	38.2	35.3
聚基线总体积平方根的改变，mm^3 mm^3	10.6	1.4

[0524] *更低的总计Agatston得分和平方根转化的总体积得分表明更低水平的血管钙化。

[0525] 表10.根据ActRIIA-Fc暴露，在总计Agatston得分方面的基线和距基线改变，排除5个逸出值*

[0526]

	ActRIIA-Fc	
	≤3 剂 n=3	>3 剂 n=7
新的基线总计 Agatston 得分，排除 5 个逸出值	1,914	3,782
距基线总计 Agatston 得分的改变，排除 5 个逸出值	148.4	274.3
% 距基线改变，排除 5 个逸出值	7.5	5.8

[0527] *更低的总计Agatston得分和平方根转化的总体积得分表明更低水平的血管钙化。

[0528] 评估了骨周转生物标记物。安慰剂组具有最高的基线处的PTH、BSAP、P1NP和CTX水平(表4)。在磷、PTH、FGF-23或硬骨素水平方面没有观察到一致的距基线改变，只是在安慰剂组中PTH在所有时间点都低于基线(-15.2到-100.7ng/L)。对于骨生物标记物，在形成标志物BSAP和BSAPP1NP方面没有见到百分比改变中的清楚变化，在再吸收标志物CTX中的百分比改变在附图11中示出。

[0529] 接受0.5mg/kgActRIIA信号转导抑制剂的更多对象在再吸收标志物CTX方面存在有意义、持续的降低。

[0530] 9.3.3 结论

[0531] 对于30名随机化的对象，13人具有成对的QCT评估(对于PBO、0.3mg/kg和0.5mg/kg分别为n＝3、6和4)。对于PBO、0.3mg/kg和0.5mg/kg距基线的相对BMD改变分别是股骨的颈部皮层的-0.9％、-1.4％和+1.9％，以及腰脊椎的+12.6％、+8.0％和1.9％。0.5mg/kg的生物标记物改变提示了有益的抗再吸收作用。

[0532] 这些数据表明，ActRIIA-Fc 0.5mg/kg的出现的剂量效应，其看起来逆转了高周转

ROD对皮层骨和松质骨的影响。PBO结果与预料相同。

[0533] 9.4 实施例4.肾脏疾病产生的循环肾脏修复因子引起心血管疾病

[0534] 肾脏疾病与极其高的死亡率相关，这与它们产生心血管疾病相关(Sarnak,M.J.et al.,2003.Circulation 108:2154-2169.)。一项进行中的REGARDS计划的辅助研究表明，在具有持续性冠状动脉事件(心脏缺血或非致命性心肌梗塞)的患者中，在4年的中值跟踪中，第二心血管事件的发生率在患有肾脏疾病的患者中是35％，相比之下高风险组(吸烟者、糖尿病、高胆固醇血症)是19％，在高风险组中各种原因的死亡发生率是慢性肾病(CKD)组的一半(Baber,U.,et al.,2013.Am Heart J 166:373-380.)。与肾脏疾病相关的提高的心血管风险的原因在于2006年命名的一种综合征——慢性肾病-骨矿物失调(CKD-MBD)(Moe,S.,et al.,2006.Kidney Int 69:1945-1953.)。在CKD-MBD中，已经发现三种新的心血管风险因素(Block,G.A.,et al.,1998.Am J Kidney Dis 31:607-617;Blacher,J.,et al.,2001.Hypertension 38:938-942;Gutierrez,O.M.,et al.,2008.New Engl J Med 359:584-592.)，它们的风险因素状态在一般群体中得到确认(Dhingra,R.,et al.,2007.Arch Intern Med 167:879-885;Matsushita,K.,et al.,2014.Journal of the American Society of Nephrology;Dalal,M.,et al.,2011.European Journal of Endocrinology 165:797-803.)。这些是高磷酸血症、血管钙化和升高的成纤维细胞生长因子23(FGF23)水平。CKD-MBD开始于CKD的早期阶段(2期)(Fang,Y.,et al.,2014.Kidney Int 85:142-150;Pereira,R.C.,et al.,2009.Bone 45:1161-1168;Fang,Y.,et al.,2009.J Am Soc Nephrol 20:36A;Hu,M.C.,et al.,2011.J Am Soc Nephrol 22:124-136.)由血管去分化/钙化、骨营养不良、Klotho损失和提高的FGF23分泌组成(Fang,Y.,et al.,2014.Kidney Int 85:142-150.)，但是在早期肾衰竭开始时进展为CKD-MBD的原因(Hu,M.C.,et al.,2011.J Am Soc Nephrol 22:124-136;Fang,Y.,et al.,2014.J Am Soc Nephrol 25:1760-1763;de Oliveira,R.B.,et al.,2013.Nephrology Dialysis Transplantation 28:2510-2517;Sabbagh,Y.2012.J.Bone Miner.R

[0535] 已经证明肾脏疾病的再激活发展程序涉及疾病刺激的肾脏修复期间的肾发生(Surendran,K.,et al.,2002.Am J Physiol Renal Physiol 282:F431-F441;Surendran,K.,et al.,2005.J Am Soc Nephrol 16:2373-2384;Maeshima,A.,et al.,2001.Cytokine&Growth Factor Reviews 12:289-298;Terada,Y.,et al.,2003.Journal of the American Society of Nephrology 14:1223-1233;Kawakami,T.,et al.,2013.J Pathol 229:221-231.)。在肾脏修复中重新激活的肾产生的因子之中，Wnt(portmanteau of Wingless and Integrated)家族对于管状上皮重构是关键的(Terada,Y.,et al.,2003.Journal of the American Society of Nephrology 14:1223-1233;Kawakami,T.,et al.,2013.J Pathol229:221-231;Rinkevich,Y.,et al.,2014.Cell Reports 7:1270-1283.).在Wnt功能的控制中，典型的信号转导转录性地诱导Wnt抑制性蛋白家族的表达，它们是分泌的蛋白，用来限制Wnt对自泌性或旁泌性因子的刺激的距离(Niida,A.,et al.,2004.Oncogene 23:8520-8526;Niehrs,C.2006.Oncogene 25:7469-7481;Reya,T.,et al.,2003.Nature 423:409-414;Chamorro,M.N.,et al.,2004.The EMBO Journal 24:73-84;Gonzalez-Sancho,J.M.,2004.Oncogene 24:1098-1103.)。Wnt抑制剂是循环的因子，Wnt抑制剂家族包括Dickkopfs(Dkk)、分泌的卷曲相关蛋白(Sfrps)、硬骨素、sostDoc 1、

crescent、Wnt抑制剂因子1(Wif1)和Icat(Niehrs,C.2006.Oncogene 25:7469-7481).Various forms of kidney disease increase renal expression of Wnt inhibitors and increase their levels in the circulation(Fang,Y.,et al.,2014.J Am Soc Nephrol 25:1760-1763;Surendran,K.,et al.,2005.J Am Soc Nephrol 16:2373-2384.)。

[0536] 在CKD的循环中升高的关键Wnt抑制剂的中和作用,Dkk1,抑制CKD诱导的血管去分化、血管钙化和肾性骨营养不良(Fang,Y.,et al.,2014.J Am Soc Nephrol 25:1760-1763.)。这种效应是令人惊讶的,因为血管平滑肌中的Wnt信号转导涉及刺激成骨细胞转变和血管钙化(Al-Aly,Z.,et al.,2007.Arteriosclerosis,Thrombosis,and Vascular Biology 27:2589-2596;Shao,J.S.,et al.,2005.Journal of Clinical Investigation 115:1210-1220.)。然而,新近的研究表明,Dkk1介导的主动脉Wnt7b的抑制刺激了smad介导的主动脉内皮-间充质转变(EnMT)和血管钙化(Cheng,S.-L.,et al.,2013.Arteriosclerosis,Thrombosis,and Vascular Biology 33:1679-1689.)。EnMT是涉及心脏瓣膜、心脏隔膜和主动脉根的发育的发育生理学的过程(Eisenberg,L.M.,and Markwald,R.R.1995.Circulation Research 77:1-6;Camenisch,T.D.,et al.,2002.Developmental Biology 248:170-181.),它可能(Zeisberg,E.M.,et al.,2007.Nat Med 13:952-961.)或可能不(Moore-Morris,T.,et al.,2014.The Journal of Clinical Investigation 124:2921-2934.)在各种成年人疾病状态中促进心脏纤维化。这一因素研究了是否有来自TGFβ超家族的其他因子涉及肾脏疾病期间的尝试肾脏修复,并在CKD期间在循环中提高。

[0537] 9.4.1 方法

[0538] 9.4.1.1 动物模型的产生

[0539] 动脉粥样硬化性低密度脂蛋白受体缺陷(ldlr-/-)的雄性(C57Bl/6J背景)购自Jackson实验室,在12周龄开始用高脂肪饮食(42%的卡路里来自脂肪)(Teklad#_)饲喂。小鼠在22周龄时是肥胖的、胰岛素抗性的,在28周龄时是糖尿病的,并且是高胆固醇血症的。

[0540] 早先描述了用来产生慢性肾病的两步过程(Davies,M.R.,et al.,2003.J Am Soc Nephrol 14:1559-1567;Davies,M.R.,et al.,2005.J Am Soc Nephrol 16:917-928.)。电灼可以在出生后12周通过2厘米的侧腹切口施用于右肾,随后在14周龄左侧进行全肾切除术。改变烧灼的强度,以产生中度的(CKD-3)肾脏损伤,其通过20周龄时的菊糖清除率来确认(附图12A)。对照动物接受假操作,其中暴露并移动适当的肾脏,但不以任何其他方式处理。五组小鼠用于这项研究(附图15B)。第一组是饲喂常规饮食的野生型C57Bl/6J小鼠(WT)。这是用于标准对照值的正常肾脏功能和饮食的组。第二组是饲喂高脂肪饮食和假操作的ldlr-/-小鼠(Sham)。这一组具有正常的肾脏的功能,它充当对照组来确定肾脏疾病的影响。第三组是具有相当于人CKD 3期的降低的GFR、饲喂高脂肪饮食的ldlr-/-小鼠(CKD-3),在22周安乐死,是基线血管钙化组(CKD-3)。第四组是在22周开始每周两次接受溶媒的皮下注射的患有CKD-3的ldlr-/-小鼠,直到第28周安乐死(CKD-3 V)。第五组是在22周开始每周两次接受10mg/kg的mActRIIA-Fc(Celgene,Summit,NJ)的皮下注射的患有CKD-3的ldlr-/-小鼠,直到第28周安乐死(CKD-3 mActRIIA-Fc)。

[0541] 使用的第二种CKD的模型是X-连锁的Alport's综合征的鼠同族体,其在IV型胶原

蛋白的α5链的基因COL4A5上是缺陷的(Rheault,M.N.,et al.,2004.Journal of the American Society of Nephrology 15:1466-1474.)。这是自发性肾脏疾病的模型,在整个结果中使用它来确认肾脏消融诱导的CKD的影响。繁育对可以购自Jackson实验室,并繁育用于实验。半合子雄性在出生后200天自发地发生与人3-4期CKD可比较的肾脏疾病。

[0542] 使用的第三种CKD模型是在用于细胞谱系追踪的转基因小鼠GNZ小鼠中的肾脏消融,类似于ldlr-/-方案(附图14)。GNZ报告物雌性小鼠(Stoller,J.Z.,et al.,2008.Genesis(New York,N.Y.2000)46:200-204.)和Tek-Cre转基因雄性小鼠(Koni,P.A.,et al.,2001.The Journal of Experimental Medicine 193:741-754.)购自Jackson实验室,并繁育产生GNZ/Tek-Cre+小鼠用于实验。GNZ/Tek-Cre-同窝出生作为阴性对照。通过使用厂家推荐用于GNZ和Tek-Cre小鼠品系的特异性引物进行小鼠基因分型。在所情况中,在麻醉下进行安乐死。腹膜腔内麻醉(甲苯噻嗪13mg/kg和氯胺酮87mg/kg)用于所有的过程。隐静脉的静脉血液样品在第二次手术后一周采集,来评估基线的术后肾脏功能。在处死时,通过心内穿刺采集血液,心脏和主动脉整体解剖。

[0543] 9.4.1.2 菊糖清除

[0544] 如果安乐死在28周,在20周或26周根据厂家指导(BioPal Inc.,Worcester,MA)进行菊糖清除。

[0545] 9.4.1.3化学的钙化定量

[0546] 在处死时解剖主动脉和心脏,在解剖显微镜下通过钝器解剖除去所有外部的组织。组织在60℃干燥20-24小时,称重,并用钵锤和研钵碾压成粉末。在4℃下在1N HCL中洗脱钙持续24小时。根据厂家的指导使用甲酚酞络合酮方法(Sigma,St Louis)分析洗脱物的钙含量,结果相对干燥组织重量来校正。

[0547] 9.4.1.4 血液测试

[0548] 通过标准的自动分析仪实验室方法,在抽血当天分析血液的血脲氮(BUN)、钙和磷酸盐。血浆激活素(Fitzgerald Industries,Acton,MA)、卵泡抑素(MYBIOSOURCE Inc.,San Diego,CA)和卵泡抑素样3(MYBIOSOURCE Inc.)水平在ELISA分析中测定。血清Dkk1用ELISA分析R&D Systems,Minn.,MN(R&D Systems,Minn.,MN)。对于ELISA分析,在安乐死时通过隐静脉或心脏穿刺抽取血液。所有血液样品在采集时置于冰上。血小板缺乏的EDTA血浆样品通过在4℃6000rpm 5分钟和14000rpm 2分钟的2步离心来制备。样品在-20℃或以下冷冻保存待用。

[0549] 9.4.1.5 组织学和免疫组织化学

[0550] 主动脉、肾脏和心脏组织固定在10%中性缓冲的福尔马林中过夜,然后转移到4℃的70%乙醇中,包埋在石蜡中,制备5微米切片。切片在二甲苯中脱石蜡,在分级的乙醇系列中脱水,然后再水化。根据标准方案,Mason Trichome染色用于检测肾脏和心脏纤维化,茜素红染色用于检测钙化(Gregory,C.A.,et al.,2004.Analytical Biochemistry 329:77-84.)。对于免疫组织化学染色,内源的过氧化物酶用甲醇中3%H_2O_2阻断15分钟。非特异性结合用抗生物素蛋白和生物素封闭试剂(Vector laboratories,Burlingame,CA,USA)阻断30分钟,然后用3%正常驴血清阻断20分钟。在用PBS洗涤是指,切片与初级抗体在4℃孵育过夜,然后与生物素化的次级抗体(Vector laboratories)在室温下孵育1小时,然后使用DAB试剂盒(SK-4100,Vector laboratories)进行链霉抗生物素蛋白结合的过氧化物酶染色。

对于双重免疫荧光染色，切片在20％山羊血清中阻断，用第一初级和次级抗体以及第二初级和次级抗体连续染色，根据厂家指导，使用山羊抗兔Alexa 488和Alexa 568次级抗体1:400(Life Technologies,A11008 and A11011)或TSA试剂盒(Life Technologies,T20932)用于信号放大。来自同一物种的初级抗体用于双重染色时，在第二次染色之前切片在柠檬酸盐缓冲液中在微波中加热5分钟(Toth and Mezey,2007)。初级抗体用于这项研究中用于免疫染色：兔多克隆抗ActRIIA抗体1:250(Abcam,ab 135634)、兔多克隆抗抑制素beta A抗体1:100(Santa Cruz,sc-50288)、兔多克隆抗CD31抗体1:50(Abcam,ab28364)和兔多克隆抗GFP抗体1:1000(Abcam,ab6556)。

[0551] 9.4.1.6 RT-PCR

[0552] 使用RNeasy Mini试剂盒(Qiagen,Valencia,CA)从主动脉和细胞培养物中提取RNA。1μg的总RNA用DNase处理，根据厂家的指导使用来自Bio-Rad(Hercules,Ca)的iScript cDNA合成试剂盒进行逆转录。使用Vector NTI(Invitrogen,Grand Island,NY)或Primer Express软件设计引物。Perkin-Elmer DNA热循环仪用于进行反应。在如上述进行逆转录之后，使用StepOne Plus实时PCR仪(AB)、来自Sigma(St.Louis)的SYBR Green和来自Invitrogen的PCR试剂盒进行实时。每个反应在95℃45秒、和60℃30秒和72℃60秒一式三份进行40个循环。随后是熔化循环，由60℃到95℃逐步提高的温度组成。在每个基因的解离曲线中观察到单一优势峰，支持了PCR产物的特异性。Ct数(阈值)在PCR的指数期内设置，用于计算目标基因的表达水平。B2m用作内标准物，用于归一化所述值。由Ct对比log cDNA稀释度(相应于log拷贝数)组成的标准曲线通过扩增与未知数量的扩增子相应的cDNA的连续稀释物来产生。通过在RT-PCR之前煮沸5分钟来确保基因组DNA不被扩增，通过灭活逆转录酶来进行阴性对照。

[0553] 9.4.1.7 免疫印迹(Western分析)

[0554] 使用含有蛋白酶抑制剂混合物(Santa Cruz)的RIPA裂解缓冲液(Thermo Scientific)，从小鼠的肾脏和主动脉制备全细胞溶胞产物蛋白.溶胞产物(20μg)装载到8-12％SDS-PAGE凝胶中，用针对抑制素β-A(Santa Cruz)、α-微管蛋白(Santa Cruz)、snai 1(Cell Signaling)、alpha-SMA(Sigma)、Runx2(Cell Signaling)、MYOCD(Santa Cruz)、ACVRL1(Origene)、ACVR1(Cell Signaling)、Erk1/2(Cell Signaling)、磷酸-Erk1/2(Cell Signaling)、ACVR1B(Origene)、Col1a1(Santa Cruz)、Smad2/3(Cell Signaling)或磷酸-Smad2/3(Cell Signaling)的抗体进行免疫印迹。在免疫沉淀(IP)分析中，使用相同的全细胞溶胞产物蛋白。为了降低非特异性结合，使用预洗涤的蛋白A琼脂糖珠子(Cell Signaling)预先清洗样品。预清洗的样品与磷酸丝氨酸抗体(Abcam)孵育过夜。然后在蛋白A琼脂糖珠子上捕获IP-抗体复合物，通过免疫印迹分析检测蛋白质。

[0555] 9.4.1.8 人类研究

[0556] 根据来自赫尔辛基声明的指南在给与知情同意之后将对象加入。如果大于18岁并患有3期CKD(估计的GFR 30-59ml/分钟/1.73m^2)使用肾病研究方程中的饮食修改(Levey,A.S.,et al.,1999.Ann Intern Med130:461-470.)，对象是合格的。排除指标包括妊娠、骨疾病、心肌梗塞、充血性心力衰竭、心脏舒张功能障碍或严重的高血压。研究组的成员确定他们的临床患者满足包含指标并对研究感兴趣。在参与之前回顾医疗记录来确定最终的合格。在基线就诊时和治疗12个月后从每个对象获得血液样品。在获得血液样品的当天存在

患者内的变异。从每份样品产生血浆等分量，直接用于生物化学测试或在-80℃冷冻。使用商业上可获得的ELISA试剂盒根据厂家的说明(R&D Systems,Minneapolis,MN)双份地测量激活素的血浆水平。

[0557] 9.4.1.9 统计

[0558] 使用ANOVA进行统计分析。所有数据表示为平均值±SD，触发在附图图例中另外指定。在使用Fisher LSD法之后评估各组之间的差异，$p<0.05$认为是显著的。使用Sigma Stat统计软件(Point Richmond,CA)进行分析。所有组的数据有7-15的“n”。对于实时PCR分析，每个实验组中使用最少3个样品。不成对的t-测验用于比较mActRIIA-Fc治疗的CKD-3小鼠和溶媒治疗的CKD-3 V小鼠或假(Sham-operated)操作的小鼠。在附图6中，方框代表中值和百分位范围(25^{th}到75^{th}百分位数)，误差线代表低于25^{th}和高于75^{th}百分位数的百分位范围的1.5倍。使用用于多重比较的ANOVA Holm-Sidak方法比较平均值，$p<0.05$作为显著差异的临界水平。

[0559] 9.4.2 结果

[0560] 9.4.2.1 CKD模型中的肾功能

[0561] 在三个实验模型中诱导类似于人3期CKD(CKD-3)的CKD。第一个模型是CKD刺激的动脉粥样硬化性血管钙化的模型：ldlr-/-高脂肪饲喂的小鼠(Fang,Y.,et al.,2014.Kidney Int 85:142-150;Fang,Y.,et al.,2014.J Am Soc Nephrol 25:1760-1763;Davies,M.R.,et al.,2003.J Am Soc Nephrol 14:1559-1567;Davies,M.R.,et al.,2005.J Am Soc Nephrol 16:917-928;Lund,R.J.,et al.,2004.J Am Soc Nephrol 15:359-369;Mathew,S.,et al.,2008.J Am Soc Nephrol 19:1092-1105.PMCID:PMC2396927;Mathew,S.,et al.,2007.J Am Soc Nephrol 18:122-130;Mathew,S.,et al.,2008.J Am Soc Nephrol 19:1509-1519.PMCID:PMC2488263.)。通过菊糖清除和通过BUN水平测量的肾脏功能在ldlr-/-高脂肪饲喂小鼠中降低到人3期CKD的水平，如章节9.4.1.1中描述的，这些小鼠此后称为CKD-3(附图12A和附图12B)。此外，CKD-3小鼠是高磷酸盐血的(表11)，这与人3b-4CKD一致。第二个CKD模型是自发性CKD，其在IV型胶原蛋白的α5链(Col4α5)缺陷(Alport's综合征)的小鼠和人中发生(Rheault,M.N.,et al.,2004.Journal of the American Society of Nephrology 15:1466-1474.)。200天龄的Col4α5缺陷小鼠具有相当于人3-4期CKD的菊糖清除降低和BUN升高(附图12C和附图12D)。第三个模型，GNZ小鼠中的CKD-3在章节9.4.1.1和下文中描述。

[0562] 表11.各组动物的血清生化参数

[0563]

参数	组 1 野生型	组 2 Sham	组 3 CKD-3	组 4 CKD-3	组 5 CKD-3
品系	C57/BJ6	*Ldlr-/-*	*Ldlr-/-*	*Ldlr-/-*	*Ldlr-/-*
饮食	常规	高脂肪	高脂肪	高脂肪	高脂肪
手术	无	假的	CKD	CKD	CKD
出生周数	28	28	28	28	28
治疗	无	无	溶媒	mActRIIA-Fc	LaC03
N	12	15	14	15	12
BUN (mg/dL)	24.0 ± 4.6	20.6 ± 3.7	37.7 ± 7.6	36.5 ± 5.8	35.6 ± 9.1
Ca (mg/di)	8.3 ± 1.8	8.9 ± 0.9	9.4 ± 0.8	8.8 ± 0.3	8.8 ± 1.4
磷 (mg/di)	8.9 ± 0.2	7.9 ± 2.3	11.0 ± 1.6	11.8 ± 1.2	10.5 ± 2.1

[0564] 9.4.2.2 激活素水平和主动脉激活素IIA型受体(ActRIIA)

[0565] 研究了CKD中的肾脏修复刺激TGFβ超家族成员的循环水平的能力。在CKD的模型中循环激活素水平提高(附图13A和附图13B)。分析肾脏和主动脉组织的激活素A(抑制素betaA的同二聚体(Inhba))的mRNA。在肾脏和主动脉中Inhba mRNA被CKD诱导(附图13C),在肾脏中蛋白质水平提高(附图13D)。分析来自患有CKD-3的小鼠的主动脉中TGFβ超家族II型受体的诱导,所述受体是由II型和I型(ALK)受体组成的超家族受体杂多聚体的配体结合部件。ldlr-/-高脂肪饲喂的小鼠中产生的CKD-3诱导了主动脉血管平滑肌中的激活素II型受体A(ActRIIA)的上调(附图13E)。与调节的抑制因子卵泡抑素和卵泡抑素样3(fstL3)相关的激活素(Welt,C.,et al.,2002.Experimental Biology and Medicine 227:724-752.),其循环水平(附图13F和附图13G)和组织水平不受CKD-3影响。在循环中卵泡抑素、fstL3和抑制素(总和620pg/ml加400pg/ml的未测出的抑制素)(Sharpe,R.M.,et al.,1999.Journal of Andrology 20:94-101.))对激活素A水平(>5000pg/ml)的化学当量表明,CKD产生了显著的游离激活素水平,是CKD中使得激活素A成为循环因子的病理事件。

[0566] 9.4.2.3 CKD中的主动脉内皮-间充质转变

[0567] CKD的第三个实验模型用于这些研究中。GNZ小鼠被用于细胞谱系追踪(Stoller,J.Z.,et al.,2008.Genesis(New York,N.Y.:2000)46:200-204.),一旦上游loxP侧翼的STOP序列被移除,GNZ小鼠表达核定位的绿色荧光蛋白和β半乳糖苷酶(GFP/LacZ)(附图14A)。为了除去GNZ小鼠中的loxP位点,它们与在酪氨酸激酶Tek(Tie2)启动子/增强子的指导下表达Cre重组酶的Tek-Cre繁育。由于Tie2是内皮谱系特异性血管生成素受体,GNZ/Tek-Cre+小鼠在内皮谱系细胞中表达核的和细胞质的GFP(附图14A)(Moore-Morris,T.,et al.,2014.The Journal of Clinical Investigation 124:2921-2934.)。在GNZ/Tek-Cre+小鼠中,与核DAPI染色共同定位的主动脉GFP限于用CD31生物标记物标记的内皮细胞(附图14B,见箭头)。当在GNZ/Tek-Cre+小鼠中产生CKD-3时,除了内皮细胞之外,主动脉中层和外膜中的细胞显现GFP染色,显示了这些细胞通过内皮-间充质转变(EnMT)来源于内皮谱系(附图14C、14D,见箭头)。为了进一步分析CKD是否刺激EnMT,在自发性CKD的模型、Alports'

s综合征小鼠中检查了涉及EnMT的转录因子Snai 1的表达。在CKD的过程中Snai 1在早期是被上调的(75doa)(附图14E),与峰值激活素水平的时机一致(见下文)。

[0568] 9.4.2.4 CKD中抑制ActRIIA信号转导的血管作用

[0569] 使用配体陷阱(附图23A),其包含与IgG1的Fc结构域融合的ActRIIA的细胞外结构域(在此称为mActRIIA-Fc)。附图23B描绘了在血管钙化模型中使用mActRIIA-Fc的实验设计,使用一治疗方案,其容许在研究mActRIIA-Fc之前八周发生CKD-3刺激的血管钙化。使用这一方案,用ActRIIA配体陷阱治疗CKD-3小鼠降低了ldlr-/-高脂肪饲喂CKD-3小鼠中CKD-3刺激的主动脉Runx2表达和主动脉碱性磷酸酶(ALP)表达(附图25A)。Runx2和ALP表达都代表了ActRIIA信号转导的可能的下游效应,主动脉中成骨细胞转变的生物标记物被mActRIIA-Fc治疗逆转。主动脉平滑肌22α(Sm22-alpha),一种分化的血管平滑肌细胞的生物标记物,被CKD-3降低,被mActRIIA-Fc刺激。CKD-3引起降低的主动脉心肌素(MYOCD)表达,是血管平滑肌细胞特异性的转录因子,但是心肌素不受mActRIIA-Fc治疗的影响。相比肾脏,已与血管钙化相关联的(Lim,K.,et al.,2012.Circulation 125:2243-2255.)主动脉Klotho表达是极低的(数据未显示)。就附图25A中CKD-3和mActRIIA-Fc治疗对mRNA的主动脉蛋白质水平的影响而言,CKD提高主动脉Runx2水平,mActRIIA-Fc归一化主动脉Runx2水平(附图25B)。CKD降低alpha平滑肌肌动蛋白(αSMA)的主动脉水平,这是分化的血管平滑肌细胞的另一种生物标记物,mActRIIA-Fc治疗提高αSMA的主动脉水平(附图25B)。心肌素水平不被CKD或mActRIIA-Fc治疗改变。

[0570] 9.4.2.5 降低的ActRIIA信号转导对血管钙化的影响

[0571] 血管钙化的CKD刺激在动脉粥样硬化和2型糖尿病的模型中研究(Al-Aly,Z.,et al.,2007.Arteriosclerosis,Thrombosis,and Vascular Biology 27:2589-2596;Towler,D.A.,et al.,1998.Journal of Biological Chemistry 273:30427-30434.),患有消融性CKD的ldlr-/-高脂肪饲喂小鼠,如上文所描述的。在CKD-3溶媒治疗的小鼠(CKD-3 V)中CKD-3引起主动脉粥样斑中钙沉积的积累(附图15A),其在用mActRIIA-Fc治疗的CKD-3小鼠(CKD-3 R)中不存在,主动脉组织钙含量降低到野生型小鼠中观察到的水平,显著低于开始mActRIIA-Fc治疗之时表现的水平,CKD-3(附图15B)。

[0572] 9.4.2.6 降低的ActRIIA信号转导对CKD刺激的心脏疾病的影响

[0573] 在血管钙化模型中研究心脏疾病的CKD刺激。CKD-3 V小鼠具有提高的心脏重量,其被mActRIIA-Fc治疗逆转(附图16A),但是不被碳酸镧治疗(CKD-3 L(Fang,Y.,et al.,2014.J Am Soc Nephrol 25:1760-1763.))逆转,CKD-3 V诱导轻微的心脏肥大(附图16B)。然而,虽然没有心脏纤维化的证据(附图16C),存在肌细胞过度生长的证据,其被mActRIIA-Fc治疗逆转。

[0574] 由于CKD中血管钙化和心脏肥大相关性的一种机制是大动脉硬化,使用早先报道的方法(Wagenseil,J.E.,et al.,2009.Circulation Research 104:1217-1224;Wagenseil,J.E.,et al.,2005.AJP-Heart and Circulatory Physiology 289:H1209-H1217.)测量了血管硬化和血压。ldlr-/-高脂肪饲喂动脉粥样硬化的小鼠中的CKD-3不产生颈动脉或主动脉的血管硬化(附图19A和19B)或血压升高(表12)。此外,在不存在提高的硬化的情况下,在用针对Dkk1的中和抗体(Fang,Y.,et al.,2014.J Am Soc Nephrol 25:1760-1763.)治疗的CKD-3小鼠中的Dkk1中和对于主动脉硬化没有作用。

[0575] 表12．28周龄小鼠的生理学心血管参数

[0576]

	WT	Sham	CKD-3 V	CKD-3 Dkk1 mAb
SBP	108.9±1.1	111.9±4.7	100.4±4.6	110.7±4.8
DBP	77.27±1.0	78.56±2.2	69.14±3.1	77.55±3.6
脉压	31.63±0.3	33.32±2.5	31.27±2.1	33.1±1.9
心率	547.1±4.7	501.2±17.4	505.6±14.3	558.6±11.2

[0577] SBP＝收缩压,DBP＝舒张压。值表示为平均值±平均标准误。每组N＝4-6只小鼠。

[0578] 9.4.2.7 主动脉和肾脏中的ActRIIA信号转导与肾脏纤维化

[0579] 通过TGFβ超家族的典型的信号转导涉及结合II型受体的配体活化它们的丝氨酸/苏氨酸激酶活性并刺激I型受体、Alk激酶的缔合与磷酸化(参见附图20中的示意图)。存在七种Alk激酶被TGFβ超家族利用,Alk4(ActRIB)是最常与激活素/ActRIIA信号转导相关的I型受体(Abe Y,et al.,2004.Growth Factors(Chur,Switzerland)22:105-110.)。在来自CKD-3小鼠模型的肾脏组织匀浆中,Alk4磷酸化没有提高,与早先的研究一致(Antsiferova,M.,and Werner,S.2012.Journal of Cell Science 125:3929-3937.)(附图18A)。相比之下,phosphosmad 2被CKD-3提高,被mActRIIA-Fc降低(附图18A),表明另一种Alk受体在CKD中肾脏激活素信号转导中的作用。在由间质性肾炎的患病的CKD-3肾脏中,Col1A1被上调,mActRIIA-Fc降低了Col1a1水平(附图18A),与由mActRIIA-Fc诱导的phosphosmad 2降低一致。此外,在CKD-3 V小鼠中诱导的肾脏Klotho表达方面存在较大的降低,其被mActRIIA-Fc矫正(附图18B)。结果,分析了在CKD-3 V中表达激活素的肾脏区室,鉴定出在管周肌成纤维细胞中的重表达,而不在上皮中表达。

[0580] 在主动脉平滑肌中,检测出Alk4和Alk1(附图18A),而没有Alk5或Alk2。Alk4和Alk1的水平不受CKD-3的影响。然而,CKD-3不提高主动脉phosphosmad 2/3水平,mActRIIA-Fc也不降低它们(附图18A)。然而,非经典ActRIIA信号转导的分析(附图20)表明,map激酶(phosphoErk1/2)既不被CKD活化也不受mActRIIA-Fc抑制,p38和JNK的血管平滑肌水平是非常低的。主动脉Actin2水平也是非常低的,其不是CKD-3刺激的。Axin2是典型的Wnt信号转导的标准生物标志物。然而,由于Dkk1是典型的Wnt信号转导的转录靶点,分析了肾脏和主动脉中的Dkk1水平作为Wnt活性的生物标志物,表明在CKD-3小鼠中mActRIIA-Fc治疗降低了肾脏Dkk1水平(附图18C),产生血浆Dkk1水平的显著降低(附图18C)。此外,主动脉Dkk1水平被CKD-3提高,被mActRIIA-Fc抑制(附图18C)。这表明肾脏和血管平滑肌的Wnt信号转导以及Dkk1的全身性释放受到激活素抑制的抑制。因而,CKD中升高的循环激活素通过提高的血管ActRIIA发信号,活化Wnt信号转导并刺激动脉粥样硬化性钙化。

[0581] 9.4.2.8 临床前研究向人类病理生理学和临床的转变

[0582] 如附图19中所示,与健康对照相比,血清激活素水平在患有3期CKD的患者群组中提高。

[0583] 9.4.3 结论

[0584] 这个实施例表明,激活素是涉及肾脏修复的第二种因子,其在CKD期间在循环中提高。不受理论的限制,结果表明,激活素表达在患病肾脏中提高,被释放到循环中,并刺激主

动脉血管平滑肌去分化和成骨细胞转变，这受到内皮向间充质转变的促进，这些作用导致CKD刺激的动脉粥样硬化性钙化和心脏肥大。CKD上调了肌成纤维细胞中的肾脏激活素表达，通过提高肾脏纤维化刺激肾脏疾病的发展。抑制ActRIIA信号转导抑制了主动脉中的成骨细胞转变和动脉粥样硬化性钙化。抑制ActRIIA信号转导抑制了心脏肥大和肾脏纤维化。ActRIIA配体陷阱mActRIIA-Fc对早期CKD-MBD综合征的作用类似于CKD刺激的血管钙化中针对Dkk1的单克隆抗体的作用(Fang,Y.,et al.,2014.J Am Soc Nephrol 25:1760-1763.)。这些数据表明，在患病肾脏中抑制ActRIIA信号转导降低了Wnt活化和循环Wnt抑制剂。此外，在血管平滑肌中，激活素刺激了Wnt活性，其受到mActRIIA-Fc的抑制。因而，两种肾脏发育因子Wnts和激活素激活素的重激活在CKD中产生了循环Wnt抑制剂和激活素，引起血管和心脏疾病。这是肾脏疾病直接导致心血管疾病的证明，肾病过程的考虑不能与肾脏修复和后续的心血管疾病刺激相分离。

[0585] 进一步的，这个实施例表明，肾脏的激活素表达在三种慢性肾病模型中提高，产生了提高的循环激活素，其超过了循环中激活素抑制剂的水平。循环激活素刺激了通过ActRIIA受体的信号转导，产生主动脉血管平滑肌去分化、成骨细胞转变和动脉粥样硬化斑块的钙化。这些效应是由于循环激活素，得到了主动脉中不存在提高的激活素蛋白质水平、而CKD诱导提高的主动脉激活素mRNA的支持(附图13B)。不受理论的限制，这些数据表明，血管的激活素信号转导通过肾脏的内分泌性激活素产生而被CKD刺激。

[0586] 由于EnMT是Smad介导的过程(Cooley,B.C.,et al.,2014.Science Translational Medicine 6:227ra234.)，被TGFβ超家族的成员，包括TGFβ、骨骼形态发生蛋白(BMP)、激活素/抑制素以及生长与分化因子(GDF)活化(Piek,E.,et al.,1999.The FASEB Journal 13:2105-2124.)，分析了患有CKD-3的血管钙化模型的主动脉中TGFβ超家族的II型受体水平的改变，其是受体的配体结合部件。激活素IIA型受体(ActRIIA)在主动脉血管平滑肌中被上调。施用与IgG1的Fc结构域的融合蛋白中的ActRIIA细胞外结构域(mActRIIA-Fc)表明，抑制ActRIIA信号转导产生了在CKD刺激动脉粥样硬化模型中主动脉平滑肌细胞中成骨细胞转变的抑制，血管平滑肌细胞基因表达的刺激，以及主动脉钙化的逆转。此外，CKD刺激了谱系跟踪小鼠模型中的EnMT。然而，激活素功能的主要位点看起来在血管平滑肌中，在此ActRIIA表达提高。

[0587] 心脏肥大，特别是左室性的，在CKD中是高度普遍的(Moran,A.,et al.,2008.American Journal of Kidney Diseases 52:839-848;Park,M.,et al.,2012.Journal of the American Society of Nephrology 23:1725-1734.)。此外，动脉粥样硬化性血管钙化模型的特征在于心脏肥大(附图16)。然而，模型中心脏肥大的产生在机制上不同于CKD刺激的血管硬化(Townsend,R.R.2015.Current Opinion in Nephrology and Hypertension 24:47-5310.1097/MNH.0000000000000086;Merx,M.W.,et al.,2005.Journal of the American Society of Nephrology 16:3357-3364.)、高血压和心脏再成型中的常见范式，高血压和心脏再成型，因为动脉粥样硬化的CKD-3小鼠(ldlr-/-高脂肪饲喂的糖尿病小鼠)不展现血管硬化也不展现高血压。由于mActRIIA-Fc在不存在FGF23水平改变的情况下矫正了心脏肥大(附图19)，CKD-3小鼠中的心脏肥大不太可能是由于FGF23(Faul,C.,et al.,2011.J Clin Invest 121:4393-4408.)。

[0588] 总之，不受理论的限制，这个实施例表明，涉及肾脏修复的第二种因子在CKD期间

在循环中提高，并引起心血管疾病。不受理论的限制，该实施例表明，激活素表达在患病肾脏中提高，被释放到循环中，并刺激主动脉血管平滑肌去分化和成骨细胞转变，这受到内皮向间充质转变的促进，这些作用导致CKD刺激的动脉粥样硬化性钙化和心脏肥大。CKD上调了肌成纤维细胞中的肾脏激活素表达，通过提高肾脏纤维化刺激肾脏疾病的发展。抑制ActRIIA信号转导抑制了主动脉中的成骨细胞转变和动脉粥样硬化性钙化。抑制ActRIIA信号转导抑制了心脏肥大和肾脏纤维化。ActRIIA配体陷阱mActRIIA-Fc对早期CKD-MBD综合征的影响显示，抑制患病肾脏中的ActRIIA信号转导降低了Wnt活化和循环Wnt抑制剂。因而，不受理论的限制，两种肾脏发育因子Wnts和激活素的重激活在CKD中产生了循环Wnt抑制剂和激活素；因而，肾脏疾病引起心血管疾病。

[0589] 9.5 实施例5:肾脏损伤/修复通过全身性WNT抑制和激活素刺激血管疾病

[0590] 9.5.1 背景

[0591] 这个实施例中呈现的结果涉及实施例4中呈现的结果(章节9.4)。

[0592] 通过在肾脏修复期间产生全身性Wnt抑制和激活素分泌，肾脏疾病导致动脉粥样硬化性血管钙化。慢性肾病(CKD)的血管效应是Wnt抑制与激活素诱导的激活素受体功能调节的相互作用。

[0593] 9.5.2 方法

[0594] 具有升高的Wnt抑制剂特别是Dkk1的CKD和激活素在谱系跟踪和血管钙化的小鼠模型中诱导，容许在Alport's小鼠中自发地发展。激活素、Dkk1、激活素2A型受体(ActRIIA)、phosphosmad 2/3和胶原蛋白水平通过ELISA、RT-PCR和Western印迹来测量。血管平滑肌功能通过压力诱导的动脉扩张术来测量。在繁育成内皮特异性Tie2-Cre小鼠的Rosa-tdT小鼠中进行细胞谱系跟踪。携带Rosa--tdT的小鼠在携带Cre重组酶的细胞中表达蕃茄红。mActRIIA-Fc是指激活素A配体陷阱(参见，例如，美国专利No.8,173,601和Carrancio et al.,2014,British Journal of Haematology,165:870-882)。

[0595] 9.5.3 结果

[0596] CKD提高循环Dkk1和激活素水平。在患病肾脏中，激活素在肌成纤维细胞中表达，通过phosphosmad 3的ActRIIA信号转导被提高。在患病肾脏中，ActRIIA配体陷阱mActRIIA-Fc抑制了肾脏的psmad 3、Col1A1表达、尿蛋白水平和Dkk1水平，并提高了Klotho水平。在血管系统中，ActRIIA水平和信号转导受到CKD的损害，伴随降低的血管平滑肌分化和功能。在CKD小鼠血管系统中，mActRIIA-Fc提高了血管平滑肌细胞分化，并抑制成骨细胞转变和血管钙化。在循环中，mActRIIA-Fc降低Dkk1水平。对比具有正常肾脏功能的Tek-Cre/Rosa-tdT小鼠，在Tek-Cre/Rosa-tdT CKD小鼠的受损的股骨动脉的外膜的细胞中CKD诱导蕃茄红表达，其中番茄红限于股动脉内皮，表明在血管损伤期间CKD诱导Tie2阳性细胞。

[0597] 9.5.4 结论

[0598] CKD降低血管平滑肌功能并刺激成骨细胞转变和血管钙化。使用mActRIIA-Fc降低CKD中升高的激活素的作用，抑制了Smad依赖性肾脏纤维化，阻断了主动脉成骨细胞转变，提高了血管平滑肌分化，并降低了血管钙化。

[0599] 9.6 实施例6:在CKD-MBD的肾性骨营养不良的病理中激活素信号转导的作用

[0600] 9.6.1.1 引言

[0601] 慢性肾病-矿物质/骨失调(CKD-MBD)包括血管钙化和骨营养不良。CKD提高循环激活素(TGFβ超家族的配体)和激活素信号转导(附图21A)。用激活素IIA型受体(ActRIIA)配体陷阱(mActRIIA-Fc)抑制ActRIIA信号转导抑制了血管钙化并防止心脏肥大。这个实施例表明激活素信号转导在肾性骨营养不良的病理中的作用。

[0602] 9.6.1.2 方法

[0603] 假操作的ldlr-/-高脂肪饲喂小鼠(SHAM;n＝12)显现糖尿病和高胆固醇血症。具有高磷酸血症、升高的FGF-23和肾小球过滤率60％降低的CKD(CKD-3)通过在ldlr-/-高脂肪饲喂小鼠中在14周龄的5/6肾切除术来诱导,是动脉粥样硬化性血管钙化的模型。CKD-3小鼠用mActRIIA-Fc 10mg/kg(mActRIIA-Fc;n＝15)或溶媒(VEH;n＝13)治疗,在22周龄开始每周腹膜内注射,在28周通过骨组织形态学和微计算机断层扫描来研究。CKD-3的结果与野生型(WT;n＝5)小鼠和SHAM比较。

[0604] 9.6.2 结果

[0605] 相对于WT(松质骨体积/组织体积(BV/TV):12.90％),SHAM小鼠展现了与衰弱性骨疾病相关的降低的BV/TV(10.92％)CKD-3的诱导在VEH小鼠中导致高周转骨疾病,和更低的BV/TV(11.22％);这被六周的mActRIIA-Fc治疗逆转(13.28％)。CKD-3的诱导引起小梁厚度降低,这被6周的mActRIIA-Fc治疗逆转。CKD-3 VEH治疗的小鼠展现了更高的侵蚀表明/骨表面和更高的破骨细胞数量/100mm骨长度(分别为1.83％和62.32/100mm),相比SHAM(1.05％和33.40/100mm),这被mActRIIA-Fc mActRIIA-Fc缓解(1.23％和38.37/100mm)。当与WT或SHAM(分别为附图21B和21C)相比时,CKD-3 VEH治疗的小鼠还导致更高的成骨细胞表面/骨表面和更高的成骨细胞数量/100mm骨长度(分别为1.58％和110.63/100mm;$p<0.05$)。与VEH相比,mActRIIA-Fc显著地降低成骨细胞表面/骨表面和成骨细胞数量/100mm骨长度(0.68％和43.18/100mm;$p<0.05$)。尽管相对于VEH在成骨细胞数量方面明显降低,用mActRIIA-Fc治疗的矿物质合并率被维持(分别为0.42和0.40μm^3/天),伴有显著更高的骨形成率/成骨细胞(分别为0.17vs.0.48μm^3/100个细胞/年;$P<0.05$vs.VEH),这类似于WT(0.42μm^3/100个细胞/年)(附图21D)。

[0606] 表13.组织形态学结果(平均+SEM)

[0607]

	Sham	CKD-3 + 溶媒	CKD-3 + mActRIIA-Fc
BV, %	10.9±1.3	11.2±0.8	13.3±1.2
小梁厚度（平），μm	30.9±2.1	31.8±1.7	33.2±1.9
小梁的分离（平板）μm	269.4±17.3	261.9±16.6	238.1±24.9
类骨质体积/BV, %	0.3±0.1	0.4±0.1	0.2±0.1
类骨质表面/骨表面%	2.5±0.6	2.9±0.7	1.7±0.4
类骨质厚度，μm	2.1±0.4	1.9±0.3	1.9±0.2
侵蚀表面/骨表面。%	1.1±0.2	1.8±0.5	1.2±0.5
破骨细胞数量/骨周长， #/100 mm	33.4±5.1	62.3±19.5	38.4±15.4
破骨细胞表面/骨表面， %	1.0±0.2	1.7±0.5	1.1±0.5
矿物质合并率/天，μm/天	0.4±0.0	0.4±0.1	0.4±0.0
双重标记/骨表面，%	3.0±0.9	3.7±1.0	1.6±0.3
单个标记/骨表面、%	8.0±0.9	10.1±1.1	10.3±1.9
矿化表面/骨表面，%	7.0±0.9	8.8±1.1	6.8±1.1
骨形成率/骨表面，mm^3/cm^2/年	10.8±2.3	13.9±2.5	9.5±1.2
矿化滞后时间，天	1.9±0.4	1.9±0.5	1.3±0.3
类骨质成熟时间，天	6.3±1.4	5.4±0.9	4.9±0.5

[0608] SEM＝平均标准误

[0609] mActRIIA-Fc不影响高磷酸血症和FGF-23水平。

[0610] 9.6.3 结论

[0611] 提高的循环激活素促进了与CKD-3相关的高周转骨营养不良。用ActRIIA配体陷阱mActRIIA-Fc抑制激活素信号转导，通过抑制骨吸收和归一化矿物质合并率以及骨形成率/成骨细胞、对抗CKD的负面效果，提高了CKD-3中的骨体积。

[0612] 9.7 实施例7：在用逐步升高的HACTRIIA-FC剂量水平治疗的血液透析对象中对骨质量和腹部主动脉血管钙化的定量计算机断层扫描结果

[0613] 9.7.1 引言

[0614] 高周转肾性骨营养不良的标志是降低的皮层骨质量和提高的小梁骨质量，引起骨折风险提高。根据体外数据，激活素II型受体-IgG1融合蛋白hActRIIA-Fc阻断了激活素A信号转导，可以降低破骨细胞发生，促进骨中的成骨细胞成熟。使用定量计算机断层扫描(QCT)在血液透析(HD)的对象中当前的分析评估了hActRIIA-Fc对骨矿物密度(BMD)和腹部主动脉血管钙化的影响。

[0615] 9.7.2 方法

[0616] 在用于矫正贫血的HD的对象中对hActRIIA-Fc的研究中，促红细胞生成素刺激剂(ESA)响应性的对象被洗出他们的ESA效应直到血红蛋白(Hb)<10g/dL，任何随机化到每28天皮下施用直到8个剂量周期的hActRIIA-Fc0.3mg/kg(n＝9)、0.5mg/kg(n＝8)、0.7mg/kg(n＝9;7例完成，2例进行中)或安慰剂(PBO;n＝9)中。利用14天剂量周期的剂量组是当前参与的。评估对象的对Hb、安全性参数、BMD、血管钙化和骨周转的生物标记物的影响。治疗失败(Hb<9g/dL)用ESA/输血来挽救。在基线和225天的治疗期之后获得髋部、腰脊椎和腹主动脉的QCT扫描。

[0617] 9.7.3 结果

[0618] 在该项研究中被随机化的35名对象中，20名具有成对的QCT评估(PBO:n＝3;0.3mg/kg:n＝6;0.5mg/kg:n＝5和0.7mg/kg:n＝6)。一些具有成对的QCT的对象由于治疗失败而对hActRIIA-Fc的暴露有限。表14展现了PBO和每个hActRIIA-Fc剂量组的BMD和血管钙化结果。与PBO相比，hActRIIA-Fc看起来缓解了高周转肾性骨营养不良对皮层和小梁BMD的影响，并减慢了血管钙化的发展。

[0619] 表14

[0620]

			hActRIIA-Fc					
	PBO	n	0.3 mg/kg	n	0.5 mg/kg	n	0.7 mg/kg	n
研究药物的天数	107.3	3	107.2	6	152.6	5	151.8	6
股骨颈部皮层BMD，平均%CFB	-0.91 %	3	-1.38	5	1.56%	5	0.90%	4
股骨颈部皮层BMD有>2%提高的对象，%	0.00%	3	20.00%	5	40.00%	5	75.00%	4
腰椎BMD(小梁的)，平均%CFB	12.59 %	3	7.95%	6	0.54%	5	1.93%	6
总计Agatston得分，平均%CFB	58.42 %	3	24.89%	6	17.26%	5	7.42%	5
Agatston得分的提高<15%的对象，%	33.33 %	3	83.33%	6	80.00%	5	100.00%	5
体积得分的平方根(mm^3)，平均CFB	4.54	3	11.07	6	1.23	5	1.26	5

[0621] CFB＝距基线改变。

[0622] 9.7.4 结论

[0623] 这些数据表明，hActRIIA-Fc以剂量依赖性的方式逆转了高周转肾性骨营养不良对皮层和小梁骨的影响，减慢了血管钙化的发展。

[0624] 9.8 实施例8.用逐步升高的ACTRIIA-HFC(SEQ ID NO:7;“SOTATERCEPT”)剂量水平治疗的血液透析对象中骨质量和腹部主动脉血管钙化的定量计算机断层扫描结果

[0625] 9.8.1 引言

[0626] 参见实施例6(章节9.7)的引言(章节9.7.1)和方法(章节9.7.2)。这个实施例呈现了在研究的较晚时间获得的、实施例6(章节9.7)中进行的研究的其他数据。

[0627] 9.8.2 结果

[0628] 总共35名对象进行随机化,接受至少一剂的安慰剂或ActRIIA-hFc(SEQ ID NO:7;"Sotatercept"),如表15中标明的。

[0629] 表15.随机化的对象和QCT分析子集合

[0630]

对象	安慰剂	Sotatercept 0.3 mg/kg	Sotatercept 0.5 mg/kg	Sotatercept 0.7 mg/kg
随机化的并接受≥1 剂量的安慰剂或Sotatercept	9	9	8	9
在基线和 225 天的 QCT 测量	4	6	5	6

[0631] 对象分布在附图22A中描绘,包括在基线和225天有成对的QCT测量的对象。停止研究治疗的大多数对象具有需要挽救的治疗识别,一般是因为血红蛋白浓度低于9g/dL。停止研究治疗的大多数对象接受了安慰剂或0.3mg/kg的sotatercept。没有对象因为有害事件停止治疗。进一步的,在具有成对的QCT测量的21名对象中,12名由于在治疗期内的血红蛋白治疗失败而需要挽救治疗,需要挽救的人中的9人处于前3个剂量周期内。

[0632] 在具有成对的QCT测量的对象中,基线的人口统计学和临床特征在治疗组之间是一般相似的(表16)。然而,在安慰剂组中存在着实质上更久的在透析时间,这也是最年轻的组。在基线生物标记物和Agatston得分方面各组之间也存在差异(表17)。

[0633] 表16.具有成对的QCT测量的对象的基线人口统计学和临床特征

[0634]

	安慰剂 (n=4)	Sotatercept 0.3 mg/kg (n=6)	Sotatercept 0.5 mg/kg (n=5)	Sotatercept 0.7 mg/kg (n=6)
年龄，平均，岁	51.3	57.3	60.8	63.0
女性，n（%）	1（25.0）	6（66.7）	0（0.0）	4（66.7）
种族，n（%）				
白人	1（25.0）	3（50.0）	3（60.0）	5（83.3）
黑人	2（50.0）	3（50.0）	2（40.0）	1（16.7）
亚洲人	1（25.0）	0（0.0）	0（0.0）	0（0.0）
族群，n（%）				
拉丁裔	0（0.0）	2（33.3）	3（60.0）	2（33.3）
非拉丁裔	4（100.0）	4（66.7）	2（40.0）	4（66.7）
透析后体重，平均， kg	65.5	80.3	79.4	84.5
体质量指数，平均， kg/m^2	24.2	27.7	26.9	29.9
糖尿病，n（%）	2（50.0）	5（83.3）	5（100.0）	5（83.3）
甲状旁腺切除， n（%）	0（0.0）	0（0.0）	0（0.0）	0（0.0）
在透析时间，平均，月	165.8	43.3	22.4	68.1
非钙磷酸盐结合剂，n（%）*	4（100.0）	3（50.0）	3（60.0）	3（50.0）
钙基磷酸盐结合剂，n（%）*	1（25.0）	4（66.7）	4（80.0）	4（66.7）
拟钙剂，n（%）*	1（25.0）	2（33.3）	0（0.0）	2（33.3）
1,25-OH 维生素 D 类似物,n（%）	3（75.0）	5（83.3）	2（40.0）	4（66.7）

[0635] *对象可以接受多种类型的结合剂

[0636] 表17.具有成对的QCT测量的对象的平均基线生物标记物、体积BMD和Agatston得分

[0637]

	安慰剂 (n=4)	Sotatercept 0.3 mg/kg (n=6)	Sotatercept 0.5 mg/kg (n=5)	Sotatercept 0.7 mg/kg (n=6)
基线整体 PTH, pg/mL	209.2	104.8	135.5	100.4
BSAP[a], μg/L	24.0	18.0	13.2	15.5
P1NP[b], ng/mL	548.3	376.2	468.4	437.5
CTX[c], pg/mL	3,152.3	2,062.5	2,266.8	2,246.5
全髋整体 BMD, mg/cm^3	276.8	286.6	280.0	281.0
股骨颈皮层 BMD, mg/cm^3	640.5	667.0	593.1	594.8
平均脊椎 (L1, L2) BMD, mg/cm^3	140.1	125.6	149.1	118.7
VC 总计 Agatston 得分[d]	6,498.8	9,472.7	3,618.5	1,862.1

[0638] 注意:n反映了具有成对的QCT评定的随机化对象的数量;对每个参数可用的对象的实际数量可能变化。PTH=甲状旁腺激素。

[0639] [a]骨=特异性碱性磷酸酶(BSAP)参考范围:男性,6-30;女性(绝经前),3-19;女性(绝经后),6-26。

[0640] [b]1型前胶原N-原肽(P1NP)参考范围:男性,30-110;女性20-108

[0641] [c]C-末端端肽(CTX)参考范围:男性,0-854,女性(绝经前),26-573;女性(绝经后),104-1,008

[0642] [d]更低的Agatston得分表明更低水平的血管钙化。

[0643] 严重有害事件一般被认为是与sotatercept不相关的,不引起停止,由继续治疗来解决。在sotatercept治疗组中没有报告死亡。有害事件在严重度上大多数是轻微的或中度的,与研究药物不相关,在治疗组之间是相对相似的,一般与对象的医学史一致。在前28天的剂量周期中和在225天治疗期期间,在任何治疗组的对象中,家庭血压测量值没有显示距基线的一致性或剂量依赖性的改变。

[0644] 腹主动脉总计Agatston得分的距基线改变在表18中提供。在腹主动脉总计Agatston得分方面有<15%进展的对象的比例在附图22B中显示。

[0645] 表18.在腹主动脉总计Agatston得分和平方根转化的总体积得分方面的基线和距基线改变*

[0646]

	安慰剂 (n=4)	Sotatercept 0.3 mg/kg (n=6)	Sotatercept 0.5 mg/kg (n=5)	Sotatercept 0.7 mg/kg (n=6)
腹主动脉总计 Agatston 得分				
基线总计 Agatston 得分	6,498.8	9,472.7	3,618.5	1,862.1
距基线总计 Agatston 得分的改变	787.8	8.578.9	225.7	171.0
%距基线总计 Agatston 得分的改变	58.4	29.9	17.3	7.4
平方根转化的总体积得分				
基线总体积平方根，mm^3	39.0	46.2	33.1	27.4
聚基线总体积平方根的改变，mm^3	3.4	11.1	1.2	1.3

[0647] 注意:n反映了具有成对的QCT评定的随机化对象的数量;对每个参数可用的对象的实际数量可能变化。

[0648] *更低的总计Agatston和平方根转化的总体积得分表明低水平的血管钙化。

[0649] 进一步的,表19提供了股骨颈部皮层和平均腰脊椎小梁体积BMD测量的距基线改变百分比。分析了在股骨颈部皮层BMD中具有>2%增进的对象的比例,确定了用sotatercept治疗与股骨颈部皮层骨的≥2%提高的对象的比例提高相关(参见表19和附图22C)。还参见Malluche et al.,2014,Clin.J.Am.Soc.Nephrol.,9:1254-1262。此外,在高周转ROD中,相比一般群体,通过腰椎小梁BMD测量的,小梁骨质量提高(即,不良质量的骨),在ESKD中在椎骨骨折率上没有降低(参见,Leonard MB,2009,Semin.Nephrol.,29:133-143,和Duan et al.,1999,J.Clin.Endocrinol.Metab.84:718-722)。在这个场景中,提高的小梁骨质量是品质不良的(参见Malluche et al,2012,J.Am.Soc.Nephrol.23:525-532。用sotatercept治疗减慢了腰椎中小梁骨质量的提高(表19)。

[0650] 表19.股骨颈部皮层和平均腰椎(L1、L2)小梁BMD的基线和距基线改变百分比

[0651]

	安慰剂 (n=4)	Sotatercept 0.3 mg/kg (n=6)	Sotatercept 0.5 mg/kg (n=5)	Sotatercept 0.7 mg/kg (n=6)
股骨颈部皮层				
基线 BMD, mg/cm^3	640.5	667.0	593.1	594.8
距基线 BMD 的改变% (n)	-1.4 (4)	-1.4 (5)	1.6 (5)	-0.1 (4)
平均腰椎 (L1, L2) 小梁				
基线 BMD, mg/cm^3	140.1	125.6	149.1	118.7
距基线 BMD 的改变% (n)	10.9 (4)	8.0 (6)	0.5 (5)	4.5 (6)

[0652] 注意:n反映了具有成对的QCT评定的随机化对象的数量;对每个参数可用的对象的实际数量可能变化。

[0653] 9.8.3 结论

[0654] 根据基线生物标志物数据,这个实施例中的对象倾向于具有高周转ROD。安慰剂组具有降低的皮层骨质量,提高的小梁骨质量和提高的血管钙化,其以与ESKD中其他大研究相似的比例发生(Raggi et al.,2011,Nephrol.Dial.Transplant.26:1327-1339),如对高周转ROD所预计的。

[0655] 在基线和225天(其是在达到八个28天剂量周期之后)测量的QCT数据表明,用sotatercept治疗高周转ROD引起了对多种CKD-MBD参数的影响,包括血管钙化的减慢的发展,提高的股骨颈部皮层骨质量,和腰脊椎骨质量的减慢的提高。血管钙化的降低和骨质量的提高与用于血管钙化的ldlr-/-高脂肪饲喂的、5/6肾切除术的小鼠模型中的组织学发现是一致的(Fang et al.,2014,Abstract,ASN Kidney Week 2014,November 11-16,2014,Philadelphia,PA)。

[0656] 9.9 实施例9:ACTRIIA-HFC(SEQ ID NO:7,"SOTATERCEPT")影响终末期肾病的多种表现

[0657] 9.9.1 背景

[0658] 这个实施例呈现了来自临床研究的数据。实施例7和8(章节9.7和章节9.8)也呈现了来自这项研究的数据。

[0659] 这项进行中的、随机、单盲、安慰剂对照的研究评估了一种用于矫正血液透析对象的贫血的ActRIIA-IgG1融合蛋白配体陷阱ActRIIA-hFc(SEQ ID NO:7;"sotatercept")的药物动力学、安全性和血红蛋白作用,并使用定量计算机断层扫描探索它对血管钙化和骨矿物密度的影响。这个实施例提供了0.3、0.5和0.7mg/kg剂量组的中间的结果。

[0660] 9.9.2 方法

[0661] 促红细胞生成素刺激剂(ESA)响应性对象洗出ESA效应直到血红蛋白<10g/dL，并随机化到每28天皮下施用安慰剂或sotatercept中持续≤8个剂量周期。治疗失败(血红蛋白<9g/dL)用ESA或输血挽救；对象内的剂量递增不被允许。在基线和225天的治疗期之后获得髋部、腰脊椎和腹主动脉的定量计算机断层扫描扫描。报告的是药物动力学、安全性、家庭血压、血红蛋白、血管钙化和骨矿物密度作用的中间结果。

[0662] 9.9.3 结果

[0663] 在用安慰剂(n＝9)或sotatercept 0.3mg/kg(n＝9)、0.5mg/kg(n＝8)和0.7mg/kg(n＝9)治疗的对象中，有害事件大多数是轻微/中度的，与研究药物不相关，在各组之间在类型/严重度方面相对相似，一般与对象的医学史一致。在安慰剂组中发生两起死亡。在家庭血压方面没有剂量依赖性改变。在225天治疗期中，在用安慰剂或sotatercept 0.3、0.5或0.7mg/kg治疗的对象中分别有33％、33％、63％和78％的血红蛋白>10g/dL。在分别用安慰剂或sotatercept 0.3、0.5或0.7mg/kg治疗的4、6、5和6名对象中获得的成对的定量计算机断层照相显示在33％、80％、80％和100％对象中有<15％的血管钙化发展，在0％、20％、40％和75％的对象中有股骨颈部皮层骨矿物密度>2％提高。

[0664] 9.9.4 结论

[0665] Sotatercept是耐受的，在血液透析中有可接受的安全性分布，在家庭血压方面没有提高。在血红蛋白、血管钙化和骨矿物密度方面存在着对sotatercept的剂量依赖性反应。

[0666] 9.10 实施例10:CKD中失调的ACTRIIA信号转导促进动脉粥样硬化性钙化和肾脏纤维化

[0667] 这个实施例涉及本文的其他实施例中描述和进行的实验，包括实施例4。

[0668] 9.10.1 方法

[0669] 9.10.1.1 动物模型的产生

[0670] 动脉粥样硬化性低密度脂蛋白受体缺陷(ldlr-/-)的雄性(C57Bl/6J背景)购自Jackson实验室，在12周龄开始饲喂高脂肪饮食(42％的卡路里来自脂肪)。小鼠在22周龄时是肥胖的、胰岛素抗性的，在28周龄时是糖尿病的，并且是高胆固醇血症的。

[0671] 早先描述了用来产生慢性肾病的两步过程(Davies MR,et al.J Am Soc Nephrol 2003;14:1559-1567;Davies MR,et al.J Am Soc Nephrol 2005;16:917-928)。电灼可以在出生后12周通过2厘米的侧腹切口施用于右肾，随后在14周龄左侧进行全肾切除术。改变烧灼的强度，以产生中度的(CKD-3)肾脏损伤，其通过20周龄时的菊糖清除来确认(附图33A、B)。对照动物接受假操作，其中暴露并移动适当的肾脏，但不以任何其他方式处理。五组小鼠用于这项研究(附图23B)。第一组是饲喂常规饮食的野生型C57Bl/6J小鼠(WT)。这是用于标准对照值的正常肾脏功能和饮食的组。第二组是饲喂高脂肪饮食和假操作的ldlr-/-小鼠(Sham)。这一组充当对照组来确定肾脏疾病的影响。第三组是具有相当于人CKD 3期的降低的GFR、饲喂高脂肪饮食的ldlr-/-小鼠(CKD-3)，在22周安乐死，是附图25A、B中使用的基线血管钙化组(CKD-3)。第四组是在22周开始每周两次接受溶媒(磷酸盐缓冲盐水)的皮下注射的患有CKD-3的ldlr-/-小鼠，直到第28周安乐死(CKD-3 V)。第五组是在22周开始每周两次接受10mg/kg的mActRIIA-Fc(Celgene,Summit,NJ)的皮下注射的患有CKD-3的ldlr-/-小鼠，直到第28周安乐死(CKD-3 R)。使用的剂量是早先在PK/PD研究中显

示对于刺激骨形成有效的剂量。

[0672] 使用的第二个CKD模型是X-连锁的Alport's综合征的鼠同族体，其在IV型胶原蛋白的α5链COL4A5的基因中是缺陷的。(Rheault MN,et al.J.the Am.Soc.Neph.2004;15:1466-1474)。这是自发性肾脏疾病的模型，在整个结果中使用它来确认肾脏消融诱导的CKD的影响。繁育对可以购自Jackson实验室，并繁育用于实验。半合子雄性在出生后150天自发地发展与人CKD 3-4期可比较的肾脏疾病，在生命第75天发展血尿症。

[0673] 在所情况中，在麻醉下进行安乐死。腹膜腔内麻醉(甲苯噻嗪13mg/kg和氯胺酮87mg/kg)用于所有的过程。隐静脉的静脉血液样品在第二次手术后一周采集，来评估基线的术后肾脏功能。在处死时，通过心内穿刺采集血液，心脏和主动脉整体解剖。

[0674] 9.10.1.2 菊糖清除

[0675] 如果安乐死在28周，在20周或26周根据厂家指导(BioPal Inc.,Worcester,MA)进行菊糖清除。

[0676] 9.10.1.3 化学的钙化定量

[0677] 在处死时解剖主动脉，在解剖显微镜下通过钝器解剖除去所有外部的组织。组织在60℃干燥20-24小时，称重，并用钵锤和研钵碾压成粉末。钙在4℃在1N HCL中洗脱24小时。根据厂家的指导使用甲酚酞络合酮方法(Sigma,St Louis,MO)分析洗脱物的钙含量，结果相对干燥组织重量来校正。

[0678] 9.10.1.4 血液测试

[0679] 通过由动物机构进行的标准自动分析仪实验室方法，在抽血当天分析血液的血脲氮(BUN)、钙和磷酸盐。血浆激活素(Fitzgerald Industries,Acton,MA)、卵泡抑素(MYBIOSOURCE Inc.,San Diego,CA)和卵泡抑素样3(MYBIOSOURCE Inc.)水平在ELISA分析中测定。血清Dkk1用ELISA分析(R&D Systems,Minn.,MN)。对于ELISA分析，在安乐死时通过隐静脉或心脏穿刺抽取血液。所有血液样品在采集时置于冰上。血小板缺乏的EDTA血浆样品通过在4℃6000rpm 5分钟和14000rpm 2分钟的2步离心来制备。样品在-20℃或以下冷冻保存待用。

[0680] 9.10.1.5 组织学和免疫组织化学

[0681] 主动脉和肾脏组织固定在10％中性缓冲的福尔马林中过夜，然后转移到4℃的70％乙醇中，包埋在石蜡中，制备5微米切片。切片在二甲苯中脱石蜡，在分级的乙醇系列中脱水，然后再水化。根据标准方案，Mason Trichome染色用于检测肾脏和心脏纤维化，茜素红染色用于检测钙化。(Gregory CA,et al.Analytical Biochem.2004;329:77-84)。对于免疫染色，切片在20％正常的山羊血清中阻断20分钟，与初级抗体在4℃孵育过夜。切片在PBS中洗涤，在20％正常的山羊血清中阻断20分钟，之后与山羊抗兔Alexa 488次级抗体1:400(Life Technologies,A11008)在室温下孵育30分钟，或根据厂家指导(Life Technologies,T 20932)使用TSA试剂盒用于信号放大。对于双重免疫荧光染色，切片在20％山羊血清中阻断，用第一初级和次级抗体以及第二初级和次级抗体连续染色，根据厂家指导，使用山羊抗兔Alexa 488和Alexa 568次级抗体1:400(Life Technologies,A11008和A11011)或TSA试剂盒(Life Technologies,T20932和T20934)用于信号放大。来自同一物种的初级抗体用于双重染色时，在第二次染色之前切片在柠檬酸盐缓冲液中在微波中加热5分钟。(Tóth ZE,J.HistoChem.&CytoChem.2007;55:545-554)。初级抗体用于这项研究中

用于免疫染色:兔多克隆抗ActRIIA抗体1:250(Abcam,ab 135634)、兔多克隆抗抑制素beta A抗体1:100(Santa Cruz,sc-50288)、兔多克隆抗CD31抗体1:50(Abcam,ab28364)和兔多克隆抗β连环蛋白抗体1:500(Abcam,ab32572)。

[0682] 9.10.1.6 RT-PCR

[0683] 使用RNeasy Mini试剂盒(Qiagen,Valencia,CA)从主动脉和肾脏中提取RNA。根据厂家的指导在Veriti Termal循环仪(Applied Biosystems)上,1μg的总RNA进行DNase处理,使用来自Bio-Rad(Hercules,Ca)的iScript cDNA合成试剂盒进行逆转录。使用Vector NTI(Invitrogen,Grand Island,NY)或Primer Express(Life Technologies,Grand Island,NY)软件设计引物。在逆转录之后,使用StepOne Plus实时PCR仪(Applied Biosystems)、来Sigma(St.Louis)的SYBR Green和来自Invitrogen的PCR试剂盒进行实时。每个反应在95℃45秒、和60℃30秒和72℃60秒一式三份进行40个循环。随后是熔化循环,由60℃到95℃逐步提高的温度组成。在每个基因的解离曲线中观察到单一优势峰,支持了PCR产物的特异性。Ct数(阈值)在PCR的指数期内设置,用于计算目标基因的表达水平。B2m用作内标准物,归一化所述值。由Ct对比log cDNA稀释度(相应于log拷贝数)组成的标准曲线通过扩增与未知数量的扩增子相应的cDNA的连续稀释物来产生。通过在RT-PCR之前煮沸5分钟来确保基因组DNA不被扩增,通过灭活逆转录酶来进行阴性对照。

[0684] 9.10.1.7 免疫印迹(Western分析)

[0685] 使用含有蛋白酶抑制剂混合物(Santa Cruz)的RIPA裂解缓冲液(Thermo Scientific),从小鼠的肾脏和主动脉制备全细胞溶胞产物蛋白.溶胞产物(20μg)装载到8-12%SDS-PAGE凝胶中,用针对抑制素β-A(Santa Cruz)、α-微管蛋白(Santa Cruz)、actin-α平滑肌(Sigma)、Runx2(Cell Signaling)、Myocd(Santa Cruz)、ACVRL1(Origene)、ACVR1(Cell Signaling)、Erk1/2(Cell Signaling)、磷酸-Erk1/2(Cell Signaling)、ACVR1B(Origene)、Col1a1(Santa Cruz)、Smad2/3(Cell Signaling)或磷酸-Smad2/3(Cell Signaling)的抗体进行免疫印迹。免疫沉淀(IP)分析使用相同的全细胞溶胞产物蛋白。为了降低非特异性结合,使用预洗涤的蛋白A琼脂糖珠子(Cell Signaling)预先清洗样品。预清洁的样品与磷酸丝氨酸抗体(Abcam)孵育过夜。然后在蛋白A琼脂糖珠子上捕获IP-抗体复合物,通过免疫印迹分析检测蛋白质。

[0686] 使用ANOVA进行统计分析。所有数据表示为平均值±SD,除非附图图例中另外指定。在使用Fisher LSD法之后评估各组之间的差异,$p<0.05$认为是显著的。所有组的数据有7-15的"n"。对于实时PCR分析,每个实验组中使用最少3个样品。对于附图26C中的血管钙化数据,方框代表中值和百分位范围(25th到75th百分位数),误差线代表低于25th和高于75th百分位数的百分位范围的1.5倍。使用用于多重比较的ANOVA Holm-Sidak方法比较中值,$p<0.05$作为显著差异的临界水平。

[0687] 9.10.2 结果

[0688] 9.10.2.1 实验设计,以及CKD模型中的肾脏

[0689] 高脂肪饲喂的ldlr-/-小鼠是动脉粥样硬化性血管钙化的模型,需要饮食和基因型两者来产生动脉粥样硬化性血管钙化。(Davies MR,et al.J Am Soc Nephrol 2003;14:1559-1567;Towler DA,et al.J.Biological Chem.1998;273:30427-30434)。通过肾脏皮层损伤和对侧肾切除,在ldlr-/-高脂肪饲喂小鼠中肾脏功能类似于人3期CKD(CKD-3)降低

(附图33A、B)。CKD-3小鼠是高磷酸盐血的(表20),这与人3b-4期CKD中的高磷酸血症发作一致。(Isakova T,et al.Kidney Int 2011;79:1370-1378)。

[0690] 表20:测试的动物中的血清生化参数

[0691]

参数	组1 野生型	组2 Sham	组3 CKD-3 V	组4 CKD-3 R
小鼠品系	C57/BJ6	Idlr-/-	Idlr-/-	Idlr-/-
饮食	正常	高脂肪	高脂肪	高脂肪
手术	无	假的	CKD	CKD
出生后周数	28	28	28	28
治疗	无	无	溶媒	mActRIIA-Fc
N	12	15	14	15
BUN (mg/dl)	24.0±4.6	20.6±3.7	37.7±7.6*	36.5±5.8*
Ca (mg/di)	8.3±1.8	8.9±0.9	9.4±0.8	8.8±0.3
磷 (mg/di)	8.9±0.2	7.9±2.3	11.0±1.6*	11.8±1.2*

[0692] *:<0.05,与组2比较的组3和4。

[0693] 9.10.2.2 CKD中的IIA型激活素受体(ActRIIA)水平

[0694] 分析来自患有CKD-3的高脂肪饲喂ldlr-/-小鼠的主动脉中的TGFβ超家族II型受体,它们是由II型和I型(ALK)受体组成的超家族受体杂多聚物的一个配体结合部件。激活素II型受体A(ActRIIA)在主动脉血管平滑肌细胞(VSMC)中表达(附图24A、B)。ldlr-/-高脂肪饲喂小鼠中产生的CKD-3诱导主动脉中的ActRIIA被下调(附图24A)。不受任何特定理论的限制,这与其他组织中报告的高循环配体水平产生的ActRIIA内在化和降解一致。(Simone ND,et al.Endocrinology 1998;139:1147-1155;Liu ZH,et al.J.Endocrinology 2006;189:409-421).通过免疫化学和免疫荧光检测没有检出内皮细胞ActRIIA(附图24B)。

[0695] 9.10.2.3 CKD中ActRIIA配体陷阱的血管作用

[0696] 在用溶媒或ActRIIA配体陷阱(mActRIIA-Fc)治疗的CKD-3小鼠的主动脉组织匀浆中分析CKD诱导的ActRIIA水平抑制的影响(附图25A、B)。通过ldlr-/-高脂肪饲喂小鼠的主动脉中Runx2和碱性磷酸酶(Alpl)的mRNA表达来评估CKD-3刺激的成骨细胞转变。CKD-3刺激了它们的表达,mActRIIA-Fc治疗逆转了CKD的影响(附图25A)。Runx2和Alpl表达都代表了主动脉中成骨细胞转变的生物标记物,其被mActRIIA-Fc治疗逆转。主动脉的平滑肌22α或转胶蛋白(Tagln)的mRNA,分化血管平滑肌细胞的生物标志物(Li L,Miano,et al.Circulation Res.1996;78:188-195),被CKD-3降低,并被mActRIIA-Fc刺激。CKD-3还引起降低的主动脉心肌素(Myocd)mRNA表达,其是血管平滑肌细胞特异性的转录因子,但是心肌素不受mActRIIA-Fc治疗的影响。就CKD-3和mActRIIA-Fc治疗对附图25A中研究的相应mRNA的主动脉蛋白质水平的影响而言,CKD提高主动脉Runx2和Alpl水平,mActRIIA-Fc归一化它们(附图25B,Alpl的数据未显示)。CKD降低Tagln和alpha平滑肌肌动蛋白(αSMA)的主动脉水平,其是分化的血管平滑肌细胞的另一种生物标志物,mActRIIA-Fc治疗提高它们它

们(附图25B,Tagln的数据未显示)。心肌素水平不被CKD或mActRIIA-Fc治疗改变。

[0697] 1dlr-/-高脂肪饲喂小鼠是动脉粥样硬化、动脉粥样硬化性钙化和2型糖尿病的模型。(Al-Aly Z,et al.Arteriosclerosis,Thrombosis,and Vascular Biol.2007;27:2589-2596;Towler DA,et al.J.Biological Chem.1998;273:30427-30434)。鉴定出的,特别是,CKD-3引起CKD-3溶媒治疗的小鼠(CKD-3 V)的主动脉粥样斑中钙沉积的积累(附图26A),并提高总组织钙水平(附图26B)。在用mActRIIA-Fc治疗的CKD-3小鼠(CKD-3mActRIIA-Fc)中不存在可见的钙沉积。此外,,mActRIIA-Fc将主动脉组织钙含量降低到如同野生型和sham小鼠中观察到的水平,以及比CKD-3组中mActRIIA-Fc治疗开始时表现的水平显著低的水平(附图26B)。

[0698] 9.10.2.4 主动脉中的ActRIIA信号转导

[0699] TGFβ超家族的典型的信号转导涉及结合II型受体的配体活化它们的丝氨酸/苏氨酸激酶活性并刺激I型受体Alk激酶的缔合与磷酸化(参见附图34中的示意图)。存在七种被TGFβ超家族利用的Alk激酶,Alk4(ActRIB)是与ActRIIA信号转导最常相关的I型受体。(Abe Y MT,et al.Growth Factors(Chur,Switzerland)2004;22:105-110)。如附图24中证明的,在从CKD-3小鼠分离的主动脉组织匀浆中ActRIIA没有降低,并且与Alk4和Alk1的降低的组织水平不相关(附图27A)。与ActRIIA信号转导相关的其他1型受体,Alk5和Alk2是不可检测的。通过测量受体杂多聚化效应(例如,调节性Smads的磷酸化)来评估ActRIIA活性。CKD-3降低主动脉phosphosmad 2/3水平(活化的smad 2/3),mActRIIA-Fc相比CKD-3V提高它们(附图27A、B)。还分析了非经典的ActRIIA信号转导(附图34):(1)map激酶(磷酸-Erk1/2)被CKD降低,没有进一步受到mActRIIA-Fc的影响(附图27A),(2)p38和JNK的血管平滑肌水平是非常低的。并且,mActRIIA-Fc p-AKT水平保持不受影响,表明主动脉AKT/PI3激酶不受ActRIIA信号转导的影响。主动脉ActRIIA信号转导(phosphosmad 3)被CKD降低,被与ActRIIA配体陷阱在动脉粥样硬化性主动脉中抑制成骨细胞转变相关的ActRIIA配体陷阱刺激(附图25A、B、C)。

[0700] 检查了另一种非典型的ActRIIA信号转导途径,Wnt途径(附图34)。Wnt信号转导显现了在主动脉内皮和VSMC之间的差异性调节。主要的典型Wnt诱导的转录因子主动脉β-连环蛋白被免疫荧光定位至内皮细胞,在VSMC中是不可检测的(附图28A、B)。分析了VSMC中的Dkk1水平作为Wnt活性的生物标志物。在CKD-3小鼠中,CKD提高VSMC Dkk1水平,mActRIIA-Fc治疗降低Dkk1水平(附图28D)。主动脉Dkk1水平被mActRIIA-Fc降低表明血管平滑肌Wnt信号转导受到ActRIIA配体陷阱的抑制。此外,如下文显示的ActRIIA配体陷阱降低肾脏Wnt活性的效应引起了循环Dkk1水平的较大的降低(附图28E)。全身性Dkk1的降低最有可能影响内皮。这引起内皮中提高的Wnt信号转导,其中beta-连环蛋白被表达,如主动脉Axin2水平的提高或显示的(附图28C)。Axin2是beta-连环蛋白刺激的即时早期基因,常用于评估Wnt信号转导。(Mao J,Wang J,Liu B,et al.Mol.Cell 2001;7:801-809)。

[0701] 9.10.2.5 ActRIIA配体陷阱对αklotho和肾脏ActRIIA信号转导的影响

[0702] 检查了肾脏αklotho水平以及CKD-3和mActRIIA-Fc治疗的影响。如附图29A所示,mActRIIA-Fc治疗诱导了肾脏αklotho水平的显著提高。检查了肾脏ActRIIA水平和ActRIIA信号转导。肾脏ActRIIA水平不受CKD-3的影响(附图29B)。主要的ActRIIA配体,激活素A,在CKD-3小鼠中被强烈地诱导,被mActRIIA-Fc治疗抑制(附图31A-D)。缺少可以的磷酸-Alk抗

体影响了I型受体活化的检测，作为替代需要磷酸丝氨酸抗体来免疫沉淀。用抗Alk抗体进行沉淀的免疫印迹。在CKD-3模型的患病肾脏中或通过mActRIIA-Fc mActRIIA-Fc治疗，Alk4磷酸化没有显著改变(附图29B)。然而，肾脏的phosphosmad 2/3被CKD-3提高，被mActRIIA-Fc降低，表明在CKD通过ActRIIA的介导中肾脏Smad 2/3活化的部件涉及不同于Alk4的Alk(附图29C)。

[0703] 检测了ActRIIA配体陷阱对肾脏纤维化的影响。附图30A-D显示了相比CKD-3V治疗的小鼠(附图30A和附图30B)，在来自CKD-3 mActRIIA-Fc治疗的小鼠三色染色的肾脏皮层切片中(附图30C和附图30D)降低的肾脏纤维化。附图35A-D显示了低放大率的冠状的肾脏切片，箭头指出了在附图30A-D中采集高功率切片的位置，箭头头部描绘了来自电灼肾脏损伤的瘢痕反应。mActRIIA-Fc降低了瘢痕反应，与激活素在伤口愈合中的作用一致。此外，mActRIIA-Fc降低了CKD-3刺激的蛋白尿(附图30E)，与mActRIIA-Fc治疗诱导肾脏phosphosmad 2/3和纤维化的降低一致。

[0704] 9.10.2.6 CKD中可能的ActRIIA配体

[0705] 存在多种可能的ActRIIA配体，包括激活素A和B，生长和分化因子11(GDF11)，骨骼形态发生蛋白9和10(BMP9和10)，以及其他BMP，例如BMP7，它们具有对受体的更低的亲和力。但是主要的ActRIIA配体是激活素。使用两种CKD模型，鉴定出全身性循环激活素A水平在ldlr-/-消融性CKD模型中有10倍升高，在Col4A5Alport's综合征小鼠模型中有5倍提高(附图31A、B)。在这些模型中，激活素B、GDF11和BMP9水平不受CKD影响。生理学上，认为在循环中存在少量游离的激活素。不受任何特定理论的限制，这可能是有感于化学计量地相等于激活素水平的抑制剂水平。激活素与循环抑制因子卵泡抑素和卵泡抑素样3(fstL3)相关(Welt C,et al.Experimental Biol.and Med.2002;227:724-752)以及与循环水平和组织水平不受CKD-3的影响或降低的抑制素相关(附图36A、B)在循环中卵泡抑素、fstL3和抑制素(例如，620pg/ml的总和，加上400pg/ml未测出的抑制素(Sharpe RM,et al.J.Andrology 1999;20:94-101)与激活素A水平(>5000pg/ml)的化学当量表明，CKD产生显著的游离激活素水平，是一种使得激活素A成为CKD中的活性循环因子的病理事件。这一数据表明，肾脏疾病产生一种或更多种循环ActRIIA配体，它们可能下调血管的ActRIIA。

[0706] 分析来自ldlr-/-动脉粥样硬化性钙化模型的肾脏组织的激活素A(抑制素betaa的同二聚体(Inhba))来鉴定提高的循环激活素的来源。在肾脏中inhba mRNA被CKD提高(附图31C)，激活素(抑制素β-A)蛋白质水平提高(附图31D)。肾脏的激活素表达定位于CKD-3小鼠的管周肌成纤维细胞中(附图32A、B)。

[0707] 9.10.3 结论

[0708] 这个实施例表明，mActRIIA-Fc提高通过磷酸化的smad2/3的水平评估的主动脉ActRIIA信号转导。此外mActRIIA-Rc治疗逆转了CKD诱导的血管平滑肌去分化、成骨细胞转变和新内膜斑块钙化。在患病肾脏中，mActRIIA-Fc抑制了，而不是刺激了ActRIIA信号转导，并降低肾脏纤维化和蛋白尿。mActRIIA-Fc治疗降低肾脏和循环Dkk1水平，表明Wnt活化在ActRIIA下游。这个实施例表明，CKD中失调的ActRIIA信号转导促进了CKD-MBD和肾脏纤维化，将ActRIIA信号转导鉴定为CKD的治疗靶点，并表明激活素配体陷阱(例如，mActRIIA-Fc)可以用于CKD的治疗。

[0709] 10.序列的说明

[0710] 表21:序列信息

[0711]

SEQ ID NO:	描述	序列
1	人ActRIIA前体多肽	MGAAAKLAFAVFLISCSSGAILGRSETQEC LFFNANWEKDRTNQTGVEPCYGDKDKRR HCFATWKNISGSIEIVKQGCWLDDINCYDR TDCVEKKDSPEVYFCCCEGNMCNEKFSYF PEMEVTQPTSNPVTPKPPYYNILLYSLVPL MLIAGIVICAFWVYRHHKMAYPPVLVPTQ DPGPPPPSPLLGLKPLQLLEVKARGRFGCV WKAQLLNEYVAVKIFPIQDKQSWQNEYEV YSLPGMKHENILQFIGAEKRGTSVDVDLW LITAFHEKGSLSDFLKANVVSWNELCHIAE TMARGLAYLHEDIPGLKDGHKPAISHRDIK SKNVLLKNNLTACIADFGLALKFEAGKSA GDTHGQVGTRRYMAPEVLEGAINFQRDAF LRIDMYAMGLVLWELASRCTAADGPVDEY MLPFEEEIGQHPSLEDMQEVVVHKKKRPV LRDYWQKHAGMAMLCETIEECWDHDAE ARLSAGCVGERITQMQRLTNIITTEDIVTV VTMVTNVDFPPKESSL
2	人ActRIIA可溶的(细胞外的),加工的多肽序列	ILGRSETQECLFFNANWEKDRTNQTGVEP CYGDKDKRRHCFATWKNISGSIEIVKQGC WLDDINCYDRTDCVEKKDSPEVYFCCCEG NMCNEKFSYFPEMEVTQPTSNPVTPKPP
3	人ActRIIA可溶的(细	ILGRSETQECLFFNANWEKDRTNQTGVEP

[0712]

SEQ ID NO:	描述	序列
	胞外的)，C-末端 15 个氨基酸删除的加工的多肽序列。	CYGDKDKRRHCFATWKNISGSIEIVKQGC WLDDINCYDRTDCVEKKDSPEVYFCCCEG NMCNEKFSYFPEM
4	编码人 ActRIIA 前体蛋白的核酸序列	ATGGGAGCTGCTGCAAAGTTGGCGTTTG CCGTCTTTCTTATCTCCTGTTCTTCAGGTG CTATACTTGGTAGATCAGAAACTCAGGAG TGTCTTTTCTTTAATGCTAATTGGGAAAA AGACAGAACCAATCAAACTGGTGTTGAA CCGTGTTATGGTGACAAAGATAAACGGC GGCATTGTTTTGCTACCTGGAAGAATATT TCTGGTTCCATTGAAATAGTGAAACAAG GTTGTTGGCTGGATGATATCAACTGCTAT GACAGGACTGATTGTGTAGAAAAAAAAG ACAGCCCTGAAGTATATTTTTGTTGCTGT GAGGGCAATATGTGTAATGAAAAGTTTTC TTATTTTCCAGAGATGGAAGTCACACAGC CCACTTCAAATCCAGTTACACCTAAGCCA CCCTATTACAACATCCTGCTCTATTCCTTG GTGCCACTTATGTTAATTGCGGGGATTGT CATTTGTGCATTTTGGGTGTACAGGCATC ACAAGATGGCCTACCCTCCTGTACTTGTT CCAACTCAAGACCCAGGACCACCCCCAC CTTCTCCATTACTAGGGTTGAAACCACTG CAGTTATTAGAAGTGAAAGCAAGGGGAA GATTTGGTTGTGTCTGGAAAGCCCAGTT GCTTAACGAATATGTGGCTGTCAAAATAT TTCCAATACAGGACAAACAGTCATGGCA AAATGAATACGAAGTCTACAGTTTGCCTG GAATGAAGCATGAGAACATATTACAGTTC ATTGGTGCAGAAAAACGAGGCACCAGTG TTGATGTGGATCTTTGGCTGATCACAGCA TTTCATGAAAAGGGTTCACTATCAGACTT TCTTAAGGCTAATGTGGTCTCTTGGAATG AACTGTGTCATATTGCAGAAACCATGGCT AGAGGATTGGCATATTTACATGAGGATAT ACCTGGCCTAAAAGATGGCCACAAACCT GCCATATCTCACAGGGACATCAAAAGTA AAAATGTGCTGTTGAAAAACAACCTGAC AGCTTGCATTGCTGACTTTGGGTTGGCCT

[0713]

SEQ ID NO:	描述	序列
		TAAAATTTGAGGCTGGCAAGTCTGCAGG CGATACCCATGGACAGGTTGGTACCCGG AGGTACATGGCTCCAGAGGTATTAGAGG GTGCTATAAACTTCGAAAGGGATGCATTT TTGAGGATAGATATGTATGCCATGGGATTA GTCCTATGGGAACTGGCTTCTCGCTGTAC TGCTGCAGATGGACCTGTAGATGAATACA TGTTGCCATTTGAGGAGGAAATTGGCCA GCATCCATCTCTTGAAGACATGCAGGAA GTTGTTGTGCATAAAAAAAAGAGGCCTG TTTTAAGAGATTATTGGCAGAAACATGCT GGAATGGCAATGCTCTGTGAAACCATTG AAGAATGTTGGGATCACGACGCAGAAGC CAGGTTATCAGCTGGATGTGTAGGTGAA AGAATTACCCAGATGCAGAGACTAACAA ATATTATTACCACAGAGGACATTGTAACA GTGGTCACAATGGTGACAAATGTTGACT TTCCTCCCAAAGAATCTAGTCTATGA
5	编码人 ActRIIA 可溶的（细胞外的）多肽的核酸序列	ATACTTGGTAGATCAGAAACTCAGGAGT GTCTTTTCTTTAATGCTAATTGGGAAAAA GACAGAACCAATCAAACTGGTGTTGAAC CGTGTTATGGTGACAAAGATAAACGGCG GCATTGTTTTGCTACCTGGAAGAATATTT CTGGTTCCATTGAAATAGTGAAACAAGG TTGTTGGCTGGATGATATCAACTGCTATG ACAGGACTGATTGTGTAGAAAAAAAAGA CAGCCCTGAAGTATATTTTTGTTGCTGTG AGGGCAATATGTGTAATGAAAAGTTTTCT TATTTTCCAGAGATGGAAGTCACACAGCC CACTTCAAATCCAGTTACACCTAAGCCAC CC
6	包含与 Fc 结构域融合的 ActRIIA 的可溶细胞外结构域的融合蛋白	THTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDX1VSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKX2VSNKALPVPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGPFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNX3HYTQKSLSLSPGK* （其

[0714]

SEQ ID NO:	描述	序列
		中 X1 是 D 或 A; X2 是 K 或 A 且 X3 是 N 或 A)
7	与人 Fc 结构域融合的人 ActRIIA 的细胞外结构域	ILGRSETQECLFFNANWEKDRTNQTGVEP CYGDKDKRRHCFATWKNISGSIEIVKQGC WLDDINCYDRTDCVEKKDSPEVYFCCCEG NMCNEKFSYFPEMEVTQPTSNPVTPKPPT GGGTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPVPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
8	蜜蜂蜂毒（mellitin，HBML）的前导序列	MKFLVNVALVFMVVYISYIYA
9	组织纤溶酶原激活物（TPA）的前导序列	MDAMKRGLCCVLLLCGAVFVSP
10	天然 ActRIIA 前导区_	MGAAAKLAFAVFLISCSSGA
11	ActRIIA-hFc 和 mActRIIA-Fc N-末端序列	ILGRSETQE
12	具有 ActRIIA 的细胞外结构域的 C-末端 15 个氨基酸删除的 ActRIIA-Fc 蛋白	ILGRSETQECLFFNANWEKDRTNQTGVEP CYGDKDKRRHCFATWKNISGSIEIVKQGC WLDDINCYDRTDCVEKKDSPEVYFCCCEG NMCNEKFSYFPEMTGGGTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPVPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
13	带有 TPA 前导序列的未加工的 ActRIIA-hFc	MDAMKRGLCCVLLLCGAVFVSPGAAILGR SETQECLFFNANWEKDRTNQTGVEPCYGD KDKRRHCFATWKNISGSIEIVKQGCWLDDI

[0715]

SEQ ID NO:	描述	序列
		NCYDRTDCVEKKDSPEVYFCCCEGNMCN EKFSYFPEMEVTQPTSNPVTPKPPTGGGTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPVPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
14	编码带有 TPA 前导序列的未加工的 ActRIIA-hFc 的核酸序列	ATGGATGCAATGAAGAGAGGGCTCTGCT GTGTGCTGCTGCTGTGTGGAGCAGTCTT CGTTTCGCCCGGCGCCGCTATACTTGGTA GATCAGAAACTCAGGAGTGTCTTTTTTTA ATGCTAATTGGGAAAAAGACAGAACCAA TCAAACTGGTGTTGAACCGTGTTATGGTG ACAAAGATAAACGGCGGCATTGTTTTGCT ACCTGGAAGAATATTTCTGGTTCCATTGA ATAGTGAAACAAGGTTGTTGGCTGGATG ATATCAACTGCTATGACAGGACTGATTGT GTAGAAAAAAAAGACAGCCCTGAAGTAT ATTTCTGTTGCTGTGAGGGCAATATGTGT AATGAAAAGTTTTCTTATTTTCCGGAGAT GGAAGTCACACAGCCCACTTCAAATCCA GTTACACCTAAGCCACCCACCGGTGGTG GAACTCACACATGCCCACCGTGCCCAGC ACCTGAACTCCTGGGGGGACCGTCAGTC TTCCTCTTCCCCCCAAAACCCAAGGACA CCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACG AAGACCCTGAGGTCAAGTTCAACTGGTA CGTGGACGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTAC AACAGCACGTACCGTGTGGTCAGCGTCC TCACCGTCCTGCACCAGGACTGGCTGAA TGGCAAGGAGTACAAGTGCAAGGTCTCC AACAAAGCCCTCCCAGTCCCCATCGAGA AAACCATCTCCAAAGCCAAAGGGCAGCC CCGAGAACCACAGGTGTACACCCTGCCC

[0716]

SEQ ID NO:	描述	序列
		CCATCCCGGGAGGAGATGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAAAGG CTTCTATCCCAGCGACATCGCCGTGGAGT GGGAGAGCAATGGGCAGCCGGAGAACA ACTACAAGACCACGCCTCCCGTGCTGGA CTCCGACGGCTCCTTCTTCCTCTATAGCA AGCTCACCGTGGACAAGAGCAGGTGGC AGCAGGGGAACGTCTTCTCATGCTCCGT GATGCATGAGGCTCTGCACAACCACTAC ACGCAGAAGAGCCTCTCCCTGTCTCCGG TAAATGAGAATTC
15	人ActRIIB可溶的(细胞外的),EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端4个氨基酸删除(SEQ ID NO:28的氨基酸25-130)、具有L79D突变的加工的多肽序列	ETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWDDD FNCYDRQECVATEENPQVYFCCCEGNFCN ERFTHLPEAGGP EVTYEPPP
16	人ActRIIB前体蛋白序列(A64)	MTAPWVALALLWGSLWPGSGRGEAETRE CIYYNANWELERTNQSGLERCEGEQDKRL HCYASWANSSGTIELVKKGCWLDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTH LPEAGGPEVTYEPPPTAPTLLTVLAYSLLPI GGLSLIVLLAFWMYRHRKPPYGHVDIHED PGPPPPSPLVGLKPLQLLEIKARGRFGCVW KAQLMNDFVAVKIFPLQDKQSWQSEREIFS TPGMKHENLLQFIAAEKRGSNLEVELWLIT AFHDKGSLTDYLKGNIITWNELCHVAETM SRGLSYLHEDVPWCRGEGHKPSIAHRDFK SKNVLLKSDLTAVLADFGLAVRFEPGKPPG DTHGQVGTRRYMAPEVLEGAINFQRDAFL RIDMYAMGLVLWELVSRCKAADGPVDEY MLPFEEEIGQHPSLEELQEVVVHKKMRPTI KDHWLKHPGLAQLCVTIEECWDHDAEAR LSAGCVEERVSLIRRSVNGTTSDCLVSLVTS

[0717]

SEQ ID NO：	描述	序列
		VTNVDLPPKESSI
17	人ActRIIB可溶的（细胞外的），加工的多肽序列（SEQ ID NO：16的氨基酸19-134）	SGRGEAETRECIYYNANWELERTNQSGLE RCEGEQDKRLHCYASWANSSGTIELVKKG CWLDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEVTYEPPPTAPT
18	人ActRIIB可溶的（细胞外的），C-末端15个氨基酸删除的加工的多肽序列（SEQ ID NO：16的氨基酸19-119）	SGRGEAETRECIYYNANWELERTNQSGLE RCEGEQDKRLHCYASWANSSGTIELVKKG CWLDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEA
19	编码人ActRIIB（A64）前体蛋白的核酸序列	ATGACGGCGCCCTGGGTGGCCCTCGCCC TCCTCTGGGGATCGCTGTGGCCCGGCTCT GGGCGTGGGGAGGCTGAGACACGGGAG TGCATCTACTACAACGCCAACTGGGAGC TGGAGCGCACCAACCAGAGCGGCCTGG AGCGCTGCGAAGGCGAGCAGGACAAGC GGCTGCACTGCTACGCCTCCTGGGCCAA CAGCTCTGGCACCATCGAGCTCGTGAAG AAGGGCTGCTGGCTAGATGACTTCAACT GCTACGATAGGCAGGAGTGTGTGGCCAC TGAGGAGAACCCCCAGGTGTACTTCTGC TGCTGTGAAGGCAACTTCTGCAACGAGC GCTTCACTCATTTGCCAGAGGCTGGGGG CCCGGAAGTCACGTACGAGCCACCCCCG ACAGCCCCCACCCTGCTCACGGTGCTGG CCTACTCACTGCTGCCCATCGGGGGCCTT TCCCTCATCGTCCTGCTGGCCTTTTGGAT GTACCGGCATCGCAAGCCCCCCTACGGT CATGTGGACATCCATGAGGACCCTGGGC CTCCACCACCATCCCCTCTGGTGGGCCTG AAGCCACTGCAGCTGCTGGAGATCAAGG CTCGGGGGCGCTTTGGCTGTGTCTGGAA GGCCCAGCTCATGAATGACTTTGTAGCTG TCAAGATCTTCCCACTCCAGGACAAGCA GTCGTGGCAGAGTGAACGGGAGATCTTC AGCACACCTGGCATGAAGCACGAGAACC

[0718]

SEQ ID NO:	描述	序列
		TGCTACAGTTCATTGCTGCCGAGAAGCG AGGCTCCAACCTCGAAGTAGAGCTGTGG CTCATCACGGCCTTCCATGACAAGGGCTC CCTCACGGATTACCTCAAGGGGAACATCA TCACATGGAACGAACTGTGTCATGTAGC AGAGACGATGTCACGAGGCCTCTCATAC CTGCATGAGGATGTGCCCTGGTGCCGTG GCGAGGGCCACAAGCCGTCTATTGCCCA CAGGGACTTTAAAAGTAAGAATGTATTGC TGAAGAGCGACCTCACAGCCGTGCTGGC TGACTTTGGCTTGGCTGTTCGATTTGAGC CAGGGAAACCTCCAGGGGACACCCACG GACAGGTAGGCACGAGACGGTACATGGC TCCTGAGGTGCTCGAGGGAGCCATCAAC TTCCAGAGAGATGCCTTCCTGCGCATTGA CATGTATGCCATGGGGTTGGTGCTGTGGG AGCTTGTGTCTCGCTGCAAGGCTGCAGA CGGACCCGTGGATGAGTACATGCTGCCCT TTGAGGAAGAGATTGGCCAGCACCCTTC GTTGGAGGAGCTGCAGGAGGTGGTGGTG CACAAGAAGATGAGGCCCACCATTAAAG ATCACTGGTTGAAACACCCGGGCCTGGC CCAGCTTTGTGTGACCATCGAGGAGTGC TGGGACCATGATGCAGAGGCTCGCTTGT CCGCGGGCTGTGTGGAGGAGCGGGTGTC CCTGATTCGGAGGTCGGTCAACGGCACT ACCTCGGACTGTCTCGTTTCCCTGGTGAC CTCTGTCACCAATGTGGACCTGCCCCCTA AAGAGTCAAGCATCTAA
20	包含与 Fc 结构域融合的 ActRIIB 的可溶的细胞外结构域(A64;SEQ ID NO: 17) 的融合蛋白	SGRGEAETRECIYYNANWELERTNQSGLE RCEGEQDKRLHCYASWANSSGTIELVKKG CWLDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEVTYEPPPTAPTG GGTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPVPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPV

[0719]

SEQ ID NO:	描述	序列
		LDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
21	包含与 Fc 结构域融合的、带有 C-末端 15 个氨基酸删除的(SEQ ID NO: 18) ActRIIB 的可溶的细胞外结构域（A64）的融合蛋白	SGRGEAETRECIYYNANWELERTNQSGLE RCEGEQDKRLHCYASWANSSGTIELVKKG CWLDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGGTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPVPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
22	人 ActRIIB 可溶的（细胞外的), EC 结构域的 N-末端 6 个氨基酸删除、EC 结构域的 C-末端 5 个氨基酸删除（SEQ ID NO: 28 的氨基酸 25-129）、具有 L79D 突变的加工的多肽序列	ETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWDDD FNCYDRQECVATEENPQVYFCCCEGNFCN ERFTHLPEAGGPEVTYEPP
23	人 ActRIIB 可溶的（细胞外的), EC 结构域的 N-末端 6 个氨基酸删除、EC 结构域的 C-末端 3 个氨基酸删除（SEQ ID NO: 28 的氨基酸 25-131）、具有 L79D 突变的加工的多肽序列	ETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWDDD FNCYDRQECVATEENPQVYFCCCEGNFCN ERFTHLPEAGGPEVTYEPPPT
24	EC 结构域的 N-末端 6 个氨基酸删除、EC 结	MDAMKRGLCCVLLLCGAVFVSPGAAETR ECIYYNANWELERTNQSGLERCEGEQDKR LHCYASWRNSSGTIELVKKGCWDDDFNCY

[0720]

SEQ ID NO：	描述	序列
	构域的 C-末端 3 个氨基酸删除（SEQ ID NO： 28 的氨基酸 25-131）、具有 L79D 突变并具有 TPA 前导序列的未加工的 ActRIIB-Fc 融合蛋白	DRQECVATEENPQVYFCCCEGNFCNERFT HLPEAGGPEVTYEPPPTGGGTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK*
25	EC 结构域的 N-末端 6 个氨基酸删除、EC 结构域的 C-末端 3 个氨基酸删除（SEQ ID NO： 28 的氨基酸 25-131）、具有 L79D 突变的加工的 ActRIIB-Fc 融合蛋白	ETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWRNSSGTIELVKKGCWDDDF NCYDRQECVATEENPQVYFCCCEGNFCNE RFTHLPEAGGPEVTYEPPPTGGGTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK*
26	人 ActRIIB 可溶的（细胞外的），加工的多肽序列（SEQ ID NO：16 的氨基酸 20-134）	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWANSSGTIELVKKGC WLDDFNCYDRQECVATEENPQVYFCCCEG NFCNERFTHLPEAGGPEVTYEPPPTAPT
27	人 ActRIIB 可溶的（细胞外的），C-末端 15 个氨基酸删除的加工的多肽序列（SEQ ID NO： 16 的氨基酸 20-119）	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWANSSGTIELVKKGC WLDDFNCYDRQECVATEENPQVYFCCCEG NFCNERFTHLPE A
28	人 ActRIIB 前体蛋白序列（R64）	MTAPWVALALLWGSLWPGSGRGEAETRE CIYYNANWELERTNQSGLERCEGEQDKRL HCYASWRNSSGTIELVKKGCWLDDFNCYD RQECVATEENPQVYFCCCEGNFCNERFTH LPEAGGPEVTYEPPPTAPTLLTVLAYSLLPI

[0721]

SEQ ID NO：	描述	序列
		GGLSLIVLLAFWMYRHRKPPYGHVDIHED PGPPPPSPLVGLKPLQLLEIKARGRFGCVW KAQLMNDFVAVKIFPLQDKQSWQSEREIFS TPGMKHENLLQFIAAEKRGSNLEVELWLIT AFHDKGSLTDYLKGNIITWNELCHVAETM SRGLSYLHEDVPWCRGEGHKPSIAHRDFK SKNVLLKSDLTAVLADFGLAVRFEPGKPPG DTHGQVGTRRYMAPEVLEGAINFQRDAFL RIDMYAMGLVLWELVSRCKAADGPVDEY MLPFEEEIGQHPSLEELQEVVVHKKMRPTI KDHWLKHPGLAQLCVTIEECWDHDAEAR LSAGCVEERVSLIRRSVNGTTSDCLVSLVTS VTNVDLPPKESSI
29	人ActRIIB可溶的（细胞外的），加工的多肽序列（SEQ ID NO：28的氨基酸19-134）	SGRGEAETRECIYYNANWELERTNQSGLE RCEGEQDKRLHCYASWRNSSGTIELVKKG CWLDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEVTYEPPPTAPT
30	人ActRIIB可溶的（细胞外的），C-末端15个氨基酸删除的加工的多肽序列（SEQ ID NO：28的氨基酸19-119）	SGRGEAETRECIYYNANWELERTNQSGLE RCEGEQDKRLHCYASWRNSSGTIELVKKG CWLDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEA
31	人ActRIIB可溶的（细胞外的），加工的多肽序列（SEQ ID NO：28的氨基酸20-134）	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWRNSSGTIELVKKGC WLDDFNCYDRQECVATEENPQVYFCCCEG NFCNERFTHLPEAGGPEVTYEPPPTAPT
32	人ActRIIB可溶的（细胞外的），C-末端15个氨基酸删除的加工的多肽序列（SEQ ID NO：28的氨基酸20-119）	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWRNSSGTIELVKKGC WLDDFNCYDRQECVATEENPQVYFCCCEG NFCNERFTHLPE A
33	人ActRIIB可溶的（细	ETRECIYYNANWELERTNQSGLERCEGEQ

[0722]

SEQ ID NO:	描述	序列
	胞外的)，EC 结构域的 N-末端 6 个氨基酸删除、EC 结构域的 C-末端 3 个氨基酸删除（SEQ ID NO: 16 的氨基酸 25-131）、具有 L79D 突变的加工的多肽序列	DKRLHCYASWANSSGTIELVKKGCWDDD FNCYDRQECVATEENPQVYFCCCEGNFCN ERFTHLPEAGGPEVTYEPPPT
34	EC 结构域的 N-末端 6 个氨基酸删除、EC 结构域的 C-末端 3 个氨基酸删除（SEQ ID NO： 16 的氨基酸 25-131)、具有 L79D 突变并具有 TPA 前导序列的未加工的 ActRIIB-Fc 融合蛋白	MDAMKRGLCCVLLLCGAVFVSPGAAETR ECIYYNANWELERTNQSGLERCEGEQDKR LHCYASWANSSGTIELVKKGCWDDDFNCY DRQECVATEENPQVYFCCCEGNFCNERFT HLPEAGGPEVTYEPPPTGGGTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK*
35	EC 结构域的 N-末端 6 个氨基酸删除、EC 结构域的 C-末端 3 个氨基酸删除（SEQ ID NO： 16 的氨基酸 25-131)、具有 L79D 突变的加工的 ActRIIB-Fc 融合蛋白	ETRECIYYNANWELERTNQSGLERCEGEQ DKRLHCYASWANSSGTIELVKKGCWDDDF NCYDRQECVATEENPQVYFCCCEGNFCNE RFTHLPEAGGPEVTYEPPPTGGGTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK*
36	人 ActRIIB 可溶的（细胞外的)，具有 L79D 突变的加工的多肽序	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWRNSSGTIELVKKGC WDDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEVTYEPPPTAPT

[0723]

SEQ ID NO：	描述	序列
	列（SEQ ID NO：28 的氨基酸 20-134）	
37	人 ActRIIB 可溶的（细胞外的），具有 L79D 突变的加工的多肽序列（SEQ ID NO：16 的氨基酸 20-134）	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWANSSGTIELVKKGC WDDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEVTYEPPPTAPT
38	人 ActRIIB 可溶的（细胞外的），具有通过 GGG 接头与 Fc 结构域融合的 L79D 突变的加工的多肽序列(SEQ ID NO： 28 的氨基酸 20-134)	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWRNSSGTIELVKKGC WDDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEVTYEPPPTAPTG GGTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK*
39	人 ActRIIB 可溶的（细胞外的），具有与 Fc 结构域融合的 L79D 突变的加工的多肽序列（SEQ ID NO：16 的氨基酸 20-134）	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWANSSGTIELVKKGC WDDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEVTYEPPPTAPTG GGTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK*
40	人 ActRIIB 可溶的（细胞外的），具有与 Fc 结构域融合的 L79D 突变和 TPA 前导序列的加工的多肽序列(SEQ ID	MDAMKRGLCCVLLLCGAVFVSPGASGRG EAETRECIYYNANWELERTNQSGLERCEG EQDKRLHCYASWRNSSGTIELVKKGCWD DDFNCYDRQECVATEENPQVYFCCCEGNF CNERFTHLPEAGGPEVTYEPPPTAPTGGGT HTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGV

[0724]

SEQ ID NO：	描述	序列
	NO： 28 的氨基酸 20-134)	EVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK*
41	人ActRIIB 可溶的（细胞外的），具有与 Fc 结构域融合的 L79D 突变和 TPA 前导序列的加工的多肽序列(SEQ ID NO： 16 的氨基酸 20-134)	MDAMKRGLCCVLLLCGAVFVSPGASGRG EAETRECIYYNANWELERTNQSGLERCEG EQDKRLHCYASWANSSGTIELVKKGCWDD DFNCYDRQECVATEENPQVYFCCCEGNFC NERFTHLPEAGGPEVTYEPPPTAPTGGGTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK*
42	人ActRIIB 可溶的（细胞外的），具有变体 C-末端序列的加工的多肽序列（WO2007/053775 中公开的）	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWRNSSGTIELVKKGC WLDDFNCYDRQECVATEENPQVYFCCCEG NFCNERFTHLPEAGGPEGPWASTTIPSGGP EATAAAGDQGSGALWLCLEGPAHE
43	人ActRIIB 可溶的（细胞外的），具有带 L79D 突变的变体 C-末端序列的加工的多肽序列（WO2007/053775 中公开的）	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWRNSSGTIELVKKGC WDDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEGPWASTTIPSGG PEATAAAGDQGSGALWLCLEGPAHE
44	人ActRIIB 可溶的（细胞外的），具有用 TGGG 接头融合到 Fc 结构域的 L79D 突变的	GRGEAETRECIYYNANWELERTNQSGLER CEGEQDKRLHCYASWRNSSGTIELVKKGC WDDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEGPWASTTIPSGG PEATAAAGDQGSGALWLCLEGPAHETGGG

[0725]

SEQ ID NO:	描述	序列
	变体 C-末端序列的加工的多肽序列（WO2007/053775 中公开的）	THTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK*
45	编码 SEQ ID NO: 24 的核酸序列	ATGGATGCAATGAAGAGAGGGCTCTGCT GTGTGCTGCTGCTGTGTGGAGCAGTCTT CGTTTCGCCCGGCGCCGCCGAAACCCGC GAATGTATTTATTACAATGCTAATTGGGAA CTCGAACGGACGAACCAATCCGGGCTCG AACGGTGTGAGGGGGAACAGGATAAAC GCCTCCATTGCTATGCGTCGTGGAGGAAC TCCTCCGGGACGATTGAACTGGTCAAGA AAGGGTGCTGGGACGACGATTTCAATTG TTATGACCGCCAGGAATGTGTCGCGACC GAAGAGAATCCGCAGGTCTATTTCTGTTG TTGCGAGGGGAATTTCTGTAATGAACGGT TTACCCACCTCCCCGAAGCCGGCGGGCC CGAGGTGACCTATGAACCCCCGCCCACC GGTGGTGGAACTCACACATGCCCACCGT GCCCAGCACCTGAACTCCTGGGGGGACC GTCAGTCTTCCTCTTCCCCCCAAAACCCA AGGACACCCTCATGATCTCCCGGACCCCT GAGGTCACATGCGTGGTGGTGGACGTGA GCCACGAAGACCCTGAGGTCAAGTTCAA CTGGTACGTGGACGGCGTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGAGGAG CAGTACAACAGCACGTACCGTGTGGTCA GCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCCAGCCCCCA TCGAGAAAACCATCTCCAAAGCCAAAGG GCAGCCCCGAGAACCACAGGTGTACACC CTGCCCCCATCCCGGGAGGAGATGACCA AGAACCAGGTCAGCCTGACCTGCCTGGT CAAAGGCTTCTATCCCAGCGACATCGCCG

[0726]

SEQ ID NO:	描述	序列
		TGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAACTACAAGACCACGCCTCCCGT GCTGGACTCCGACGGCTCCTTCTTCCTCT ATAGCAAGCTCACCGTGGACAAGAGCAG GTGGCAGCAGGGGAACGTCTTCTCATGC TCCGTGATGCATGAGGCTCTGCACAACC ACTACACGCAGAAGAGCCTCTCCCTGTC CCCGGGTAAATGA
46	包含与 Fc 结构域融合的 ActRIIB 的可溶的细胞外结构域(R64;SEQ ID NO: 29) 的融合蛋白	SGRGEAETRECIYYNANWELERTNQSGLE RCEGEQDKRLHCYASWRNSSGTIELVKKG CWLDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGPEVTYEPPPTAPTG GGTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPVPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
47	包含与 Fc 结构域融合的、带有 C-末端 15 个氨基酸删除的(SEQ ID NO: 30) ActRIIB 的可溶的细胞外结构域 (R64) 的融合蛋白	SGRGEAETRECIYYNANWELERTNQSGLE RCEGEQDKRLHCYASWRNSSGTIELVKKG CWLDDFNCYDRQECVATEENPQVYFCCCE GNFCNERFTHLPEAGGGTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPVPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
48	Runx2 qRT-PCR 引物 1	CCGCACGACAACCGCACCAT
49	Runx2 qRT-PCR 引物 2	CGCTCCGGCCCACAAATCTC
50	Alp qRT-PCR 引物 1	ACGTGGCTAAGAATGTCATC
51	Alp qRT-PCR 引物 2	CTGGTAGGCGATGTCCTTA
52	osterix qRT-PCR 引物 1	TAGTGGTTTGGGGTTTGTTTACCGC
53	osterix qRT-PCR 引物 2	AACCAAATAACTCTTATTCCCTAAGT

[0727]

SEQ ID NO:	描述	序列
54	Klotho qRT-PCR 引物 1	GCTCTCAAAGCCCACATACTG
55	Klotho qRT-PCR 引物 1	GCAGCATAACGATAGAGGCC
56	Sm22-alpha qRT-PCR 引物 1	GTTCCAGACTGTTGACCTCTTT
57	Sm22-alpha qRT-PCR 引物 2	CTGCGCTTTCTTCATAAACC
58	人类 Runx2 mRNA	GTGTGAATGCTTCATTCGCCTCACAAACA ACCACAGAACCACAAGTGCGGTGCAAA CTTTCTCCAGGAGGACAGCAAGAAGTCT CTGGTTTTTAAATGGTTAATCTCCGCAGG TCACTACCAGCCACCGAGACCAACAGAG TCATTTAAGGCTGCAAGCAGTATTTACAA CAGAGGGTACAAGTTCTATCTGAAAAAA AAAGGAGGGACTATGGCATCAAACAGCC TCTTCAGCACAGTGACACCATGTCAGCA AAACTTCTTTTGGGATCCGAGCACCAGC CGGCGCTTCAGCCCCCCCTCCAGCAGCC TGCAGCCCGGCAAAATGAGCGACGTGAG CCCGGTGGTGGCTGCGCAACAGCAGCAG CAACAGCAGCAGCAGCAACAGCAGCAG CAGCAGCAGCAACAGCAGCAGCAGCAG CAGGAGGCGGCGGCGGCGGCTGCGGCG GCGGCGGCGGCTGCGGCGGCGGCAGCT GCAGTGCCCCGGTTGCGGCCGCCCCACG ACAACCGCACCATGGTGGAGATCATCGC CGACCACCCGGCCGAACTCGTCCGCACC GACAGCCCCAACTTCCTGTGCTCGGTGC TGCCCTCGCACTGGCGCTGCAACAAGAC CCTGCCCGTGGCCTTCAAGGTGGTAGCC CTCGGAGAGGTACCAGATGGGACTGTGG TTACTGTCATGGCGGGTAACGATGAAAAT TATTCTGCTGAGCTCCGGAATGCCTCTGC TGTTATGAAAAACCAAGTAGCAAGGTTC AACGATCTGAGATTTGTGGGCCGGAGTG GACGAGGCAAGAGTTTCACCTTGACCAT AACCGTCTTCACAAATCCTCCCCAAGTAG CTACCTATCACAGAGCAATTAAAGTTACA GTAGATGGACCTCGGGAACCCAGAAGGC

[0728]

SEQ ID NO:	描述	序列
		ACAGACAGAAGCTTGATGACTCTAAACC TAGTTTGTTCTCTGACCGCCTCAGTGATT TAGGGCGCATTCCTCATCCCAGTATGAGA GTAGGTGTCCCGCCTCAGAACCCACGGC CCTCCCTGAACTCTGCACCAAGTCCTTTT AATCCACAAGGACAGAGTCAGATTACAG ACCCCAGGCAGGCACAGTCTTCCCCGCC GTGGTCCTATGACCAGTCTTACCCCTCCT ACCTGAGCCAGATGACGTCCCCGTCCAT CCACTCTACCACCCCGCTGTCTTCCACAC GGGGCACTGGGCTTCCTGCCATCACCGA TGTGCCTAGGCGCATTTCAGATGATGACA CTGCCACCTCTGACTTCTGCCTCTGGCCT TCCACTCTCAGTAAGAAGAGCCAGGCAG GTGCTTCAGAACTGGGCCCTTTTTCAGA CCCCAGGCAGTTCCCAAGCATTTCATCCC TCACTGAGAGCCGCTTCTCCAACCCACG AATGCACTATCCAGCCACCTTTACTTACA CCCCGCCAGTCACCTCAGGCATGTCCCTC GGTATGTCCGCCACCACTCACTACCACAC CTACCTGCCACCACCCTACCCCGGCTCTT CCCAAAGCCAGAGTGGACCCTTCCAGAC CAGCAGCACTCCATATCTCTACTATGGCA CTTCGTCAGGATCCTATCAGTTTCCCATG GTGCCGGGGGGAGACCGGTCTCCTTCCA GAATGCTTCCGCCATGCACCACCACCTCG AATGGCAGCACGCTATTAAATCCAAATTT GCCTAACCAGAATGATGGTGTTGACGCT GATGGAAGCCACAGCAGTTCCCCAACTG TTTTGAATTCTAGTGGCAGAATGGATGAA TCTGTTTGGCGACCATATTGAAATTCCTC AGCAGTGGCCCAGTGGTATCTGGGGGCC ACATCCCACACGTATCAATATATACATATA TAGAGAGAGTGCATATATATGTATATCGAT TAGCTATCTACAAAGTGCCTATTTTTTAGA AGATTTTTCATTCACTCACTCAGTCATGA TCTTGCAGCCATAAGAGGGTAGATATTGA GAAGCAGAAGGCTCAAGAGAGACAATT GCAATCGAGCTTCAGATTGTTTACTATTT AAGATGTACTTTTACAAAGGAACAAAGA

[0729]

SEQ ID NO:	描述	序列
		AGGGAAAAGGTATTTTTGTTTTTGTTGTT TGGTCTGTTATCATCAATAACCTGTTCATA TGCCAATTCAGAGAGGTGGACTCCAGGT TCAGGAGGGAGAAGAGCAAAGCCGCTT CCTCTCTGTGCTTTGAAACTTCACACCCT CACGGTGGCAGCTGTGTATGGACCAGTG CCCTCCGCAGACAGCTCACAAAACCAGT TGAGGTGCACTAAAGGGACATGAGGTAG AATGGATGCTTCCATCACAGTACCATCAT TCAGAATAACTCTTCCAATTTCTGCTTTC AGACATGCTGCAGGTCCTCATCTGAACT GTTGGGTTCGTTTTTTTTTTTTTTTCCT GCTCCAAGAAAGTGACTTCAAAAATAAC TGATCAGGATAGATTATTTTATTTTACTTT TTAACACTCCTTCTCCCCTTTTCCCACTG AACCAAAAAGAAATCCCATCCCTAAAAC CTGCCTTCTCCTTTTATGCAAAACTGAAA ATGGCAATACATTATTATAGCCATAATGGT ATAGATAGTGATTGCGTTTGGCTATGTGTT GTTTTCTTTTTTTTTAAATTATGAATATGT GTAAAATCTGAGGTAACTTGCTAACGTG AATGGTCATATAACTTTAAAGATATATTTA TAATTATTTAATGACATTTGGACCCTTGAA ACATTTCTTAGTGTATTGATATGTTGACTT CGGTCTCTAAAAGTGCTCTTTATTAAATA ACAAATTTCTTCAGTGGTCTAGAGCCATA TCTGAAATATTGCTAAGCAATTTCAGTTC ATCCAGGCACAATGTGATTTTAAAAAATA CTTCCATCTCCAAATATTTTAGATATAGAT TGTTTTTGTGATGTATGAAGGAAATGTTA TGTTTAGTTCTTTCAGATCTTTGAATGCCT CTAACACAGCTTTGCCTTCTAAAGCGGTA ATTAGGGATTTAAAAAACAACCTTTAGCC CTTTATCAGCATGAAATGCTGGAGTGATG TGGTTTTCTAATTTCTTTGGGGTAATTATG ACTCTTGTCATATTAAAAAGACAAGCACA AGTAAATCATTGAACTACAGAAAAATGTT CTGTGGTTTCATAGTTAAGCAAAACTCTA AATCGCCAGGCTTCATAGCAAAGACATA GTCAGCTAAAAGCCGCACATGTGGATAG

[0730]

SEQ ID NO:	描述	序列
		AGGGTTCAATTATGAGACACCTAGTACAG GAGAGCAAAATTGCACCAGAGATTCTTA ACCAACCAGCCTTACCAAACAACACAAC AGGGGAACCCCAATCTGCCTTACCCAAG GCCCCACTGGCAGCTTTCCACAGAATTT GCATTTAGAGGAGCAGAATGACATCACT GTCCTTTGGGAGTAGGTCCTCTGAAAAG GCAGCAGGTTCCAGCAGGTAGCTGAGCT GAGAGGACATATGGCCCACGGGGACCTA CAGACAGCCTTTGACATTTGTATTTCTTA CAATGGAGGGCCAAGGAGGGCAAGGGG CTGTGGAGTTTGGTGTCTACTAGTGTGTA TGAATTTGAGCTAGAGTCCTTCTGTGGCA TGCACTTTGACCACTCCTGGCAGTCACAT GGCAGATTTCCAAGTGCAAATCCTTAATC CAAACAAGGATCATCTAATGACACCACC AGGCCAATCCCTGCTCTCCTCCCCGAAA AGTCAGGGTCCCTTCATTGGAATCCTCCA CCCACCCAAGCAGAATTTAGCAGAGATT TGCCTTCAAACCCTAACGGCCCCCTTGTT CTCTGGTCCTTCTCAAACCCACCTTTGTA GGCCACCCAGCATTGCAGGACAGCGTGT GGGGCAGCTGGACCTGTGCTTCCTGCCT GGGAGTCTCCCTTGGAATTCATCCTGACT CCTTCTAATAAAAATGGATGGGAAAGCA AAACACTTTGCCTTCTAAAGGCCGTATAC CAAGTATGCTTAGATAAATAAGCCACTTT TCTATTACTTAAGTAAGAAGGAAGTAGTA ATTGATACTATTTATTGTTTGTGTGGTA GCTTGAAGCACACCACTGTCCATTTATTT GTAAGTGTAAAATATGTGTGTTTGTTTCA GCAGCACTTAAAAAAGCCAGTGTCTGGT TACACATTTCAATTTTAATTAATTGACATA AAAATGCTACCGCCAGTGCCAGCTGCAT CCTATTTAATTAAAAAGGTACTATATTTGT ACATTATTTTTTAATGTTAAAAGGGCTTTT TTAAGTTTACAGTACACATACCGAGTGAC TTTAGGGATGCTTTTGTGTTGAAATGTTA CTATAGTGGCTGCAGGCAGCAACCCAGA AACACTTTAGAAGCTTTTTTTCCTTGGGA

[0731]

SEQ ID NO:	描述	序列
		AAAATTCAAGCACTTCTTCCCTCCACCCT CACTCCAACCACCCCAATGGGGGTAATTC ACATTTCTTAGAACAAATTCTGCCCTTTT TTGGTCTAGGGATTAAAATTTTGTTTTTCT TTCTTTCTTTTTTTTTTTTTTTCACTGAAC CCTTAATTTGCACTGGGTCATGTGTTTGA TTTGTGATTTCAAGACCAAAGCAAAGTC TTACTACTACTGTGGAACCATGTACTAGT TCCTGGGAATTAAAATAGCGTGGTTCTCT TTGTAGCACAAACATTGCTGGAATTTGCA GTCTTTTCAATGCAGCCACATTTTTATCCA TTTCAGTTGTCTCACAAATTTTAACCCATA TCAGAGTTCCAGAACAGGTACCACAGCT TTGGTTTTAGATTAGTGGAATAACATTCA GCCCAGAACTGAGAAACTCAACAGATTA ACTATCGTTTGCTCTTTAGACGGTCTCAC TGCCTCTCACTTGCCAGAGCCCTTTCAAA ATGAGCAGAGAAGTCCACACCATTAGGG ACCATCTGTGATAAATTCAGAAGGGAGG AGATGTGTGTACAGCTTTAAGGATTCCCT CAATTCCGAGGAAAGGGACTGGCCCAGA ATCCAGGTTAATACATGGAAACACGAAG CATTAGCAAAAGTAATAATTATACCTATGG TATTTGAAAGAACAATAATAAAAGACACT TCTTCCAAACCTTGAATTTGTTGTTTTTA GAAAACGAATGCATTTAAAAATATTTTCT ATGTGAGAATTTTTTAGATGTGTGTTTACT TCATGTTTACAAATAACTGTTTGCTTTTTA ATGCAGTACTTTGAAATATATCAGCCAAA ACCATAACTTACAATAATTTCTTAGGTATT CTGAATAAAATTCCATTTCTTTTGGATATG CTTTACCATTCTTAGGTTTCTGTGGAACA AAAATATTTGTAGCATTTTGTGTAAATACA AGCTTTCATTTTTATTTTTTCCAATTGCTA TTGCCCAAGAATTGCTTTCCATGCACATA TTGTAAAAATTCCGCTTTGTGCCACAGGT CATGATTGTGGATGAGTTTACTCTTAACT TCAAAGGGACTATTTGTATTGTATGTTGC AACTGTAAATTGAATTATTTGGCATTTTTC TCATGATTGTAATATTAATTTGAAGTTTGA

[0732]

SEQ ID NO:	描述	序列
		ATTTAATTTTCAATAAAATGGCTTTTTTGG TTTTGTTA
59	人类 Alp mRNA	CCGGGCCTCACTCGGGCCCCGCGGCCGC CTTTATAAGGCGGCGGGGGTGGTGGCCC GGGCCGCGTTGCGCTCCCGCCACTCCGC GCCCGCTATCCTGGCTCCGTGCTCCCACG CGCTTGTGCCTGGACGGACCCTCGCCAG TGCTCTGCGCAGGATTGGAACATCAGTTA ACATCTGACCACTGCCAGCCCACCCCCT CCCACCCACGTCGATTGCATCTCTGGGCT CCAGGGATAAAGCAGGTCTTGGGGTGCA CCATGATTTCACCATTCTTAGTACTGGCCA TTGGCACCTGCCTTACTAACTCCTTAGTG CCAGAGAAAGAGAAAGACCCCAAGTAC TGGCGAGACCAAGCGCAAGAGACACTG AAATATGCCCTGGAGCTTCAGAAGCTCA ACACCAACGTGGCTAAGAATGTCATCATG TTCCTGGGAGATGGGATGGGTGTCTCCA CAGTGACGGCTGCCCGCATCCTCAAGGG TCAGCTCCACCACAACCCTGGGGAGGAG ACCAGGCTGGAGATGGACAAGTTCCCCT TCGTGGCCCTCTCCAAGACGTACAACAC CAATGCCCAGGTCCCTGACAGCGCCGGC ACCGCCACCGCCTACCTGTGTGGGGTGA AGGCCAATGAGGGCACCGTGGGGGTAAG CGCAGCCACTGAGCGTTCCCGGTGCAAC ACCACCCAGGGGAACGAGGTCACCTCCA TCCTGCGCTGGGCCAAGGACGCTGGGAA ATCTGTGGGCATTGTGACCACCACGAGA GTGAACCATGCCACCCCCAGCGCCGCCT ACGCCCACTCGGCTGACCGGGACTGGTA CTCAGACAACGAGATGCCCCCTGAGGCC TTGAGCCAGGGCTGTAAGGACATCGCCT ACCAGCTCATGCATAACATCAGGGACATT GACGTGATCATGGGGGGTGGCCGGAAAT ACATGTACCCCAAGAATAAAACTGATGTG GAGTATGAGAGTGACGAGAAAGCCAGG GGCACGAGGCTGGACGGCCTGGACCTCG TTGACACCTGGAAGAGCTTCAAACCGAG

[0733]

SEQ ID NO:	描述	序列
		ATACAAGCACTCCCACTTCATCTGGAACC GCACGGAACTCCTGACCCTTGACCCCCA CAATGTGGACTACCTATTGGGTCTCTTCG AGCCAGGGGACATGCAGTACGAGCTGAA CAGGAACAACGTGACGGACCCGTCACTC TCCGAGATGGTGGTGGTGGCCATCCAGA TCCTGCGGAAGAACCCCAAAGGCTTCTT CTTGCTGGTGGAAGGAGGCAGAATTGAC CACGGGCACCATGAAGGAAAAGCCAAG CAGGCCCTGCATGAGGCGGTGGAGATGG ACCGGGCCATCGGGCAGGCAGGCAGCTT GACCTCCTCGGAAGACACTCTGACCGTG GTCACTGCGGACCATTCCCACGTCTTCAC ATTTGGTGGATACACCCCCCGTGGCAACT CTATCTTTGGTCTGGCCCCCATGCTGAGT GACACAGACAAGAAGCCCTTCACTGCCA TCCTGTATGGCAATGGGCCTGGCTACAAG GTGGTGGGCGGTGAACGAGAGAATGTCT CCATGGTGGACTATGCTCACAACAACTAC CAGGCGCAGTCTGCTGTGCCCCTGCGCC ACGAGACCCACGGCGGGGAGGACGTGG CCGTCTTCTCCAAGGGCCCCATGGCGCA CCTGCTGCACGGCGTCCACGAGCAGAAC TACGTCCCCCACGTGATGGCGTATGCAGC CTGCATCGGGGCCAACCTCGGCCACTGT GCTCCTGCCAGCTCGGCAGGCAGCCTTG CTGCAGGCCCCCTGCTGCTCGCGCTGGC CCTCTACCCCCTGAGCGTCCTGTTCTGAG GGCCCAGGGCCCGGGCACCCACAAGCCC GTGACAGATGCCAACTTCCCACACGGCA GCCCCCCCCTCAAGGGGCAGGGAGGTGG GGGCCTCCTCAGCCTCTGCAACTGCAAG AAAGGGGACCCAAGAAACCAAAGTCTG CCGCCCACCTCGCTCCCCTCTGGAATCTT CCCCAAGGGCCAAACCCACTTCTGGCCT CCAGCCTTTGCTCCCTCCCCGCTGCCCTT TGGCCAACAGGGTAGATTTCTCTTGGGC AGGCAGAGAGTACAGACTGCAGACATTC TCAAAGCCTCTTATTTTTCTAGCGAACGT ATTTCTCCAGACCCAGAGGCCCTGAAGC

[0734]

SEQ ID NO:	描述	序列
		CTCCGTGGAACATTCTGGATCTGACCCTC CCAGTCTCATCTCCTGACCCTCCCACTCC CATCTCCTTACCTCTGGAACCCCCCAGGC CCTACAATGCTCATGTCCCTGTCCCCAGG CCCAGCCCTCCTTCAGGGGAGTTGAGGT CTTTCTCCTCAGGACAAGGCCTTGCTCAC TCACTCACTCCAAGACCACCAGGGTCCC AGGAAGCCGGTGCCTGGGTGGCCATCCT ACCCAGCGTGGCCCAGGCCGGGAAGAG CCACCTGGCAGGGCTCACACTCCTGGGC TCTGAACACACACGCCAGCTCCTCTCTG AAGCGACTCTCCTGTTTGGAACGGCAAA AAAAAATTTTTTTTCTCTTTTTGGTGGT GGTTAAAAGGGAACACAAAACATTTAAA TAAAACTTTCCAAATATTTCCGAGGACAA AAAAAAAAA
60	人类 Osterix mRNA	CGGCGGGCGGCAGCAGCCTAGGCAGCA GCAGTAGCAGAAGCAGCAGCCGCCGAG CAGCAGCAAGGACTCTGGAGTCAGAGTA GGACTGTAGGACCGGAGCCTGAGTGGAA CAGGAGTGGAGCTGGCCTGGGAGAGAG CGGATCCCTCCCAGCACCCTCAGGCCAC CCGTTGCCTGCACTCTCCCTGCCAGACCT CCAGAGAGGAGAGACTCGGGACAGCCA GCCCCAGGTTCCCCCAGCTCTCTCCATCT GCCTGGCTCCTTGGGACCCGTTCCCCAG CCTCAGGATGGCGTCCTCCCTGCTTGAG GAGGAAGTTCACTATGGCTCCAGTCCCCT GGCCATGCTGACGGCAGCGTGCAGCAAA TTTGGTGGCTCTAGCCCTCTGCGGGACTC AACAACTCTGGGCAAAGCAGGCACAAA GAAGCCGTACTCTGTGGGCAGTGACCTT TCAGCCTCCAAAACCATGGGGGATGCTTA TCCAGCCCCCTTTACAAGCACTAATGGGC TCCTTTCACCTGCAGGCAGTCCTCCAGC ACCCACCTCAGGCTATGCTAATGATTACC CTCCCTTTTCCCACTCATTCCCTGGGCCC ACAGGCACCCAGGACCCTGGGCTACTAG TGCCCAAGGGGCACAGCTCTTCTGACTG

[0735]

SEQ ID NO:	描述	序列
		TCTGCCCAGTGTCTACACCTCTCTGGACA TGACACACCCCTATGGCTCCTGGTACAAG GCAGGCATCCATGCAGGCATTTCACCAG GCCCAGGCAACACTCCTACTCCATGGTG GGATATGCACCCTGGAGGCAACTGGCTA GGTGGTGGGCAGGGCCAGGGTGATGGGC TGCAAGGGACACTGCCCACAGGTCCAGC TCAGCCTCCACTGAACCCCCAGCTGCCC ACCTACCCATCTGACTTTGCTCCCCTTAA TCCAGCCCCCTACCCAGCTCCCCACCTCT TGCAACCAGGGCCCCAGCATGTCTTGCC CCAAGATGTCTATAAACCCAAGGCAGTG GGAAATAGTGGGCAGCTAGAAGGGAGTG GTGGAGCCAAACCCCCACGGGGTGCAA GCACTGGGGGTAGTGGTGGATATGGGGG CAGTGGGGCAGGGCGCTCCTCCTGCGAC TGCCCTAATTGCCAGGAGCTAGAGCGGC TGGGAGCAGCAGCGGCTGGGCTGCGGA AGAAGCCCATCCACAGCTGCCACATCCC TGGCTGCGGCAAGGTGTATGGCAAGGCT TCGCACCTGAAGGCCCACTTGCGCTGGC ACACAGGCGAGAGGCCCTTCGTCTGCAA CTGGCTCTTCTGCGGCAAGAGGTTCACT CGTTCGGATGAGCTGGAGCGTCATGTGC GCACTCACACCCGGGAGAAGAAGTTCAC CTGCCTGCTCTGCTCCAAGCGCTTTACCC GAAGCGACCACCTGAGCAAACACCAGC GCACCCATGGAGAACCAGGCCCGGGTCC CCCTCCCAGTGGCCCCAAGGAGCTGGGG GAGGGCCGCAGCACGGGGGAAGAGGAG GCCAGTCAGACGCCCCGACCTTCTGCCT CGCCAGCAACCCCAGAGAAAGCCCCTGG AGGCAGCCCTGAGCAGAGCAACTTGCTG GAGATCTGAGCCGGGTGGAAGGTCTCCC ACCCCAGGGCTGCCCTGACAGTCTCTCT TGGCTCTCTAGACCACTGCTTGCCAATCA CTCTCTTTACCCCATGCATGCCATCCTTCG GGGCTCTCTCCCTCTGTCTCCCTCCTGGC CATTCTGGGCTTGGGTATCTCCTTGCATG CCTCCTCAGCTCACCTTCTCTCTTCACCA

[0736]

SEQ ID NO:	描述	序列
		TGAGACTGGCTTTCCACAAACTCTCATCT CAGGCCCTCCCCTTGTGCCTGATACCTGC ACTCCGGCTTCCTAGACTCTGGCCCTGCC ACACCAACACACTTTCTATTTGGGCTCCC AACACTATTTCTCCATCTCACTCCTTGAC ATGTACCCCTTTCTGCTTCTCAAGCTTATT TCCTGCTGTCCCTCAGCCTCCAGGCTTCA GTCTTCCCAACTTCTTACACCATTGCTTT CCATTCTCCAGAACTCTTTTTTCCTTTTTA CAAACACAATGATAATGATAATTTATTGC CCCCTGGTGGCCTCTTCATCAGGGGTATT GGGGTTAGTGACCTGGCCAGAGGGTGCC AAGAGGGGGGCAGACCAGTGGGGATCT GATCCCAAAGATGGGGTGACCCCAGGGT CAGGGAGGCTGCCCCCAGGCCTGTATATT TAACCCCTATGTACCAGGAGTAATGAATA GTAATAATTCTATTTATGTAAGTTATGATG ACGGGTCAGGTAGAGTGAGCTGGGGAG GGAAGTGGATCCATTTCTGCTAAGGAAAT TCTAGTCAAATGCATCTCTGTATAGACAA AATGTTAGTGGAGAAGATCTTGTTAATAG AATGTCTATCATCAGAATCTCAGTTGATA GGGTTTCTCTTGTAATGAAGTCTCTACAA ATTGGGTTAGCTACATCTCTGCTAAACAG TTGATGGGGTATCTCTTGATTAGGGGGAT CCCTAATATCCCCAGCCCCAGCCAGAAGC TGTGAAACCTCAAGTCCTATGGAGGGGA GAAGGACTGGAATGTACCCCATCTCCCTT GACTGCAGAGCAGGTTCCTCCACTGCCC CACCCCTTAGACACCATGACCCCATCAGG TTAATCCCCTGTTGCCATGGTTATGGAGA GCTTGCAGCTGCCATCTTAGATGTGCTCT TTGGGGAAGCCCATCTAACAGGAGGACA TTGGTTTGGGGGTGCACCTCCTGAAGAA TGGGTGGGGAAGGCTTTCTCTAGGATCA GATTCAAATAAGTATGTATTGAGTGCCTA CTCTGTGCAAGGCACTATGCTAGATCTGG TGCCTAGAAGCCCTGAGAAAGAACTTAA AGAGCTAGGAGGACAGAGGCCCCCAAG CTGATCTGGTGGTGCATCCACGCACCCCC

[0737]

SEQ ID NO:	描述	序列
		ACCCTGGGACTTTGGATGCTCCCATCTCC ACCTCCAGTGACTTTTAAAGCCGCTTCGT GCCTTTCCTGTAACGTTGGATCCTCCTTT TCTGTCCCCTGCTGTCTCAAGGCCCCAA GTTAAAGGGTTAAAGCCGCTGGAGCTTG GGGAGAGAACATTGTGGAATGGAAGGGA TCATGCCCTTTGTGGAGTCTTTTTTTTTA ATTTAATAAATAAAAGTTGGATTTGAAAA AAAAAAAAAAAAAAAAAAAA
61	人类 Klotho mRNA	CGCGCAGCATGCCCGCCAGCGCCCCGCC GCGCCGCCCGCGGCCGCCGCCGCCGTCG CTGTCGCTGCTGCTGGTGCTGCTGGGCCT GGGCGGCCGCCGCCTGCGTGCGGAGCCG GGCGACGGCGCGCAGACCTGGGCCCGTG TCTCGCGGCCTCCTGCCCCCGAGGCCGC GGGCCTCTTCCAGGGCACCTTCCCCGAC GGCTTCCTCTGGGCCGTGGGCAGCGCCG CCTACCAGACCGAGGGCGGCTGGCAGCA GCACGGCAAGGGTGCGTCCATCTGGGAC ACGTTCACCCACCACCCCCTGGCACCCC CGGGAGACTCCCGGAACGCCAGTCTGCC GTTGGGCGCCCCGTCGCCGCTGCAGCCC GCCACCGGGGACGTAGCCAGCGACAGCT ACAACAACGTCTTCCGCGACACGGAGGC GCTGCGCGAGCTCGGGGTCACTCACTAC CGCTTCTCCATCTCGTGGGCGCGAGTGCT CCCCAATGGCAGCGCGGGCGTCCCCAAC CGCGAGGGGCTGCGCTACTACCGGCGCC TGCTGGAGCGGCTGCGGGAGCTGGGCGT GCAGCCCGTGGTCACCCTGTACCACTGG GACCTGCCCCAGCGCCTGCAGGACGCCT ACGGCGGCTGGGCCAACCGCGCCCTGGC CGACCACTTCAGGGATTACGCGGAGCTC TGCTTCCGCCACTTCGGCGGTCAGGTCA AGTACTGGATCACCATCGACAACCCCTAC GTGGTGGCCTGGCACGGCTACGCCACCG GGCGCCTGGCCCCCGGCATCCGGGGCAG CCCGCGGCTCGGGTACCTGGTGGCGCAC AACCTCCTCCTGGCTCATGCCAAAGTCTG

[0738]

SEQ ID NO:	描述	序列
		GCATCTCTACAATACTTCTTTCCGTCCCA CTCAGGGAGGTCAGGTGTCCATTGCCCT AAGCTCTCACTGGATCAATCCTCGAAGA ATGACCGACCACAGCATCAAAGAATGTC AAAAATCTCTGGACTTTGTACTAGGTTGG TTTGCCAAACCCGTATTTATTGATGGTGA CTATCCCGAGAGCATGAAGAATAACCTTT CATCTATTCTGCCTGATTTTACTGAATCTG AGAAAAAGTTCATCAAAGGAACTGCTGA CTTTTTTGCTCTTTGCTTTGGACCCACCT TGAGTTTTCAACTTTTGGACCCTCACATG AAGTTCCGCCAATTGGAATCTCCCAACCT GAGGCAACTGCTTTCCTGGATTGACCTTG AATTTAACCATCCTCAAATATTTATTGTGG AAAATGGCTGGTTTGTCTCAGGGACCAC CAAGAGAGATGATGCCAAATATATGTATT ACCTCAAAAAGTTCATCATGGAAACCTTA AAAGCCATCAAGCTGGATGGGGTGGATG TCATCGGGTATACCGCATGGTCCCTCATG GATGGTTTCGAGTGGCACAGAGGTTACA GCATCAGGCGTGGACTCTTCTATGTTGAC TTTCTAAGCCAGGACAAGATGTTGTTGCC AAAGTCTTCAGCCTTGTTCTACCAAAAG CTGATAGAGAAAAATGGCTTCCCTCCTTT ACCTGAAAATCAGCCCCTAGAAGGGACA TTTCCCTGTGACTTTGCTTGGGGAGTTGT TGACAACTACATTCAAGTAGATACCACTC TGTCTCAGTTTACCGACCTGAATGTTTAC CTGTGGGATGTCCACCACAGTAAAAGGC TTATTAAAGTGGATGGGGTTGTGACCAAG AAGAGGAAATCCTACTGTGTTGACTTTGC TGCCATCCAGCCCCAGATCGCTTTACTCC AGGAAATGCACGTTACACATTTTCGCTTC TCCCTGGACTGGGCCCTGATTCTCCCTCT GGGTAACCAGTCCCAGGTGAACCACACC ATCCTGCAGTACTATCGCTGCATGGCCAG CGAGCTTGTCCGTGTCAACATCACCCCA GTGGTGGCCCTGTGGCAGCCTATGGCCC CGAACCAAGGACTGCCGCGCCTCCTGGC CAGGCAGGGCGCCTGGGAGAACCCCTAC

[0739]

SEQ ID NO:	描述	序列
		ACTGCCCTGGCCTTTGCAGAGTATGCCCG ACTGTGCTTTCAAGAGCTCGGCCATCAC GTCAAGCTTTGGATAACGATGAATGAGCC GTATACAAGGAATATGACATACAGTGCTG GCCACAACCTTCTGAAGGCCCATGCCCT GGCTTGGCATGTGTACAATGAAAAGTTTA GGCATGCTCAGAATGGGAAAATATCCATA GCCTTGCAGGCTGATTGGATAGAACCTGC CTGCCCTTTCTCCCAAAAGGACAAAGAG GTGGCCGAGAGAGTTTTGGAATTTGACA TTGGCTGGCTGGCTGAGCCCATTTTCGGC TCTGGAGATTATCCATGGGTGATGAGGGA CTGGCTGAACCAAAGAAACAATTTTCTT CTTCCTTATTTCACTGAAGATGAAAAAAA GCTAATCCAGGGTACCTTTGACTTTTTGG CTTTAAGCCATTATACCACCATCCTTGTAG ACTCAGAAAAAGAAGATCCAATAAAATA CAATGATTACCTAGAAGTGCAAGAAATG ACCGACATCACGTGGCTCAACTCCCCCA GTCAGGTGGCGGTAGTGCCCTGGGGGTT GCGCAAAGTGCTGAACTGGCTGAAGTTC AAGTACGGAGACCTCCCCATGTACATAAT ATCCAACGGAATCGATGACGGGCTGCAT GCTGAGGACGACCAGCTGAGGGTGTATT ATATGCAGAATTACATAAACGAAGCTCTC AAAGCCCACATACTGGATGGTATCAATCT TTGCGGATACTTTGCTTATTCGTTTAACG ACCGCACAGCTCCGAGGTTTGGCCTCTAT CGTTATGCTGCAGATCAGTTTGAGCCCAA GGCATCCATGAAACATTACAGGAAAATTA TTGACAGCAATGGTTTCCCGGGCCCAGA AACTCTGGAAAGATTTTGTCCAGAAGAA TTCACCGTGTGTACTGAGTGCAGTTTTTT TCACACCCGAAAGTCTTTACTGGCTTTCA TAGCTTTTCTATTTTTTGCTTCTATTATTTC TCTCTCCCTTATATTTTACTACTCGAAGAA AGGCAGAAGAAGTTACAAATAGTTCTGA ACATTTTTCTATTCATTCATTTTGAAATAA TTATGCAGACACATCAGCTGTTAACCATT TGCACCTCTAAGTGTTGTGAAACTGTAAA

[0740]

SEQ ID NO:	描述	序列
		TTTCATACATTTGACTTCTAGAAAACATTT TTGTGGCTTATGACAGAGGTTTTGAAATG GGCATAGGTGATCGTAAAATATTGAATAA TGCGAATAGTGCCTGAATTTGTTCTCTTT TTGGGTGATTAAAAAACTGACAGGCACT ATAATTTCTGTAACACACTAACAAAAGCA TGAAAAATAGGAACCACACCAATGCAAC ATTTGTGCAGAAATTTGAATGACAAGATT AGGAATATTTTCTTCTGCACCCACTTCTA AATTTAATGTTTTTCTGGAAGTAGTAATTG CAAGAGTTCGAATAGAAAGTTATGTACC AAGTAACCATTTCTCAGCTGCCATAATAA TGCCTAGTGGCTTCCCCTCTGTCAAATCT AGTTTCCTATGGAAAAGAAGATGGCAGA TACAGGAGAGACGACAGAGGGTCCTAGG CTGGAATGTTCCTTTCGAAAGCAATGCTT CTATCAAATACTAGTATTAATTTATGTATCT GGTTAATGACATACTTGGAGAGCAAATTA TGGAAATGTGTATTTTATATGATTTTTGAG GTCCTGTCTAAACCCTGTGTCCCTGAGGG ATCTGTCTCACTGGCATCTTGTTGAGGGC CTTGCACATAGGAAACTTTTGATAAGTAT CTGCGGAAAAACAAACATGAATCCTGTG ATATTGGGCTCTTCAGGAAGCATAAAGCA ATTGTGAAATACAGTATACCGCAGTGGCT CTAGGTGGAGGAAAGGAGGAAAAAGTG CTTATTATGTGCAACATTATGATTAATCTG ATTATACACCATTTTTGAGCAGATCTTGG AATGAATGACATGACCTTTCCCTAGAGAA TAAGGATGAAATAATCACTCATTCTATGA ACAGTGACACTACTTTCTATTCTTTAGCT GTACTGTAATTTCTTTGAGTTGATAGTTTT ACAAATTCTTAATAGGTTCAAAAGCAATC TGGTCTGAATAACACTGGATTTGTTTCTG TGATCTCTGAGGTCTATTTTATGTTTTTGC TGCTACTTCTGTGGAAGTAGCTTTGAACT AGTTTTACTTTGAACTTTCACGCTGAAAC ATGCTAGTGATATCTAGAAAGGGCTAATT AGGTCTCATCCTTTAATGCCCCTTAAATA AGTCTTGCTGATTTTCAGACAGGGAAGT

[0741]

SEQ ID NO:	描述	序列
		CTCTCTATTACACTGGAGCTGTTTTATAGA TAAGTCAATATTGTATCAGGCAAGATAAA CCAATGTCATAACAGGCATTGCCAACCTC ACTGACACAGGGTCATAGTGTATAATAAT ATACTGTACTATATAATATATCATCTTTAGA GGTATGATTTTTTCATGAAAGATAAGCTT TTGGTAATATTCATTTTAAAGTGGACTTAT TAAAATTGGATGCTAGAGAATCAAGTTTA TTTTATGTATATATTTTTCTGATTATAAGAG TAATATATGTTCATTGTAAAAATTTTTAAA ACACAGAAACTATATGCAAAGAAAAAAT AAAAATTATCTATAATCTCAGAACCCAGA AATAGCCACTATTAACATTTCCTACGTATT TTATTTTACATAGATCATATTGTATATAGTT AGTATCTTTATTAATTTTTATTATGAAACTT TCCTTTGTCATTATTAGTCTTCAAAAGCAT GATTTTTAATAGTTGTTGAGTATTCCACCA CAGGAATGTATCACAACTTAACCGTTCCC GTTTGTTAGACTAGTTTCTTATTAATGTTG ATGAATGTTGTTTAAAAATAATTTTGTTGC TACATTTACTTTAATTTCCTTGACTGTAAA GAGAAGTAATTTTGCTCCTTGATAAAGTA TTATATTAATAATAAATCTGCCTGCAACTT TTTGCCTTCTTTCATAATC
62	人类 Sm22-alpha mRNA	TCACCACGGCGGCAGCCCTTTAAACCCC TCACCCAGCCAGCGCCCCATCCTGTCTGT CCGAACCCAGACACAAGTCTTCACTCCT TCCTGCGAGCCCTGAGGAAGCCTTGTGA GTGCATTGGCTGGGGCTTGGAGGGAAGT TGGGCTGGAGCTGGACAGGAGCAGTGG GTGCATTTCAGGCAGGCTCTCCTGAGGT CCCAGGCGCCAGCTCCAGCTCCCTGGCT AGGGAAACCCACCCTCTCAGTCAGCATG GGGGCCCAAGCTCCAGGCAGGGTGGGCT GGATCACTAGCGTCCTGGATCTCTCTCAG ACTGGGCAGCCCCGGGCTCATTGAAATG CCCCGGATGACTTGGCTAGTGCAGAGGA ATTGATGGAAACCACCGGGGTGAGAGGG AGGCTCCCCATCTCAGCCAGCCACATCC

[0742]

SEQ ID NO:	描述	序列
		ACAAGGTGTGTGTAAGGGTGCAGGCGCC GGCCGGTTAGGCCAAGGCTCTACTGTCT GTTGCCCCTCCAGGAGAACTTCCAAGGA GCTTTCCCCAGACATGGCCAACAAGGGT CCTTCCTATGGCATGAGCCGCGAAGTGCA GTCCAAAATCGAGAAGAAGTATGACGAG GAGCTGGAGGAGCGGCTGGTGGAGTGG ATCATAGTGCAGTGTGGCCCTGATGTGGG CCGCCCAGACCGTGGGCGCTTGGGCTTC CAGGTCTGGCTGAAGAATGGCGTGATTC TGAGCAAGCTGGTGAACAGCCTGTACCC TGATGGCTCCAAGCCGGTGAAGGTGCCC GAGAACCCACCCTCCATGGTCTTCAAGC AGATGGAGCAGGTGGCTCAGTTCCTGAA GGCGGCTGAGGACTATGGGGTCATCAAG ACTGACATGTTCCAGACTGTTGACCTCTT TGAAGGCAAAGACATGGCAGCAGTGCA GAGGACCCTGATGGCTTTGGGCAGCTTG GCAGTGACCAAGAATGATGGGCACTACC GTGGAGATCCCAACTGGTTTATGAAGAA AGCGCAGGAGCATAAGAGGGAATTCACA GAGAGCCAGCTGCAGGAGGGAAAGCAT GTCATTGGCCTTCAGATGGGCAGCAACA GAGGGGCCTCCCAGGCCGGCATGACAGG CTACGGACGACCTCGGCAGATCATCAGTT AGAGCGGAGAGGGCTAGCCCTGAGCCCG GCCCTCCCCCAGCTCCTTGGCTGCAGCCA TCCCGCTTAGCCTGCCTCACCCACACCCG TGTGGTACCTTCAGCCCTGGCCAAGCTTT GAGGCTCTGTCACTGAGCAATGGTAACT GCACCTGGGCAGCTCCTCCCTGTGCCCC CAGCCTCAGCCCAACTTCTTACCCGAAA GCATCACTGCCTTGGCCCCTCCCTCCCGG CTGCCCCCATCACCTCTACTGTCTCCTCC CTGGGCTAAGCAGGGGAGAAGCGGGCT GGGGGTAGCCTGGATGTGGGCCAAGTCC ACTGTCCTCCTTGGCGGCAAAAGCCCAT TGAAGAAGAACCAGCCCAGCCTGCCCCC TATCTTGTCCTGGAATATTTTTGGGGTTGG AACTCAAAAAAAAAAAAAAAAAATCAA

[0743]

SEQ ID NO:	描述	序列
		TCTTTTCTCAAAAAAAAAAAAAAAAA
63	人类 Runx2 蛋白	MASNSLFSTVTPCQQNFFWDPSTSRRFSPP SSSLQPGKMSDVSPVVAAQQQQQQQQQQ QQQQQQQQQQQQQEAAAAAAAAAAAAAAA AAAAVPRLRPPHDNRTMVEIIADHPAELVR TDSPNFLCSVLPSHWRCNKTLPVAFKVVAL GEVPDGTVVTVMAGNDENYSAELRNASA VMKNQVARFNDLRFVGRSGRGKSFTLTIT VFTNPPQVATYHRAIKVTVDGPREPRRHR QKLDDSKPSLFSDRLSDLGRIPHPSMRVGV PPQNPRPSLNSAPSPFNPQGQSQITDPRQA QSSPPWSYDQSYPSYLSQMTSPSIHSTTPLS STRGTGLPAITDVPRRISDDDTATSDFCLWP STLSKKSQAGASELGPFSDPRQFPSISSLTES RFSNPRMHYPATFTYTPPVTSGMSLGMSAT THYHTYLPPPYPGSSQSQSGPFQTSSTPYLY YGTSSGSYQFPMVPGGDRSPSRMLPPCTTT SNGSTLLNPNLPNQNDGVDADGSHSSSPT VLNSSGRMDESVWRPY
64	人类 Alp 蛋白	MISPFLVLAIGTCLTNSLVPEKEKDPKYWR DQAQETLKYALELQKLNTNVAKNVIMFLG DGMGVSTVTAARILKGQLHHNPGEETRLE MDKFPFVALSKTYNTNAQVPDSAGTATAY LCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSA AYAHSADRDWYSDNEMPPEALSQGCKDIA YQLMHNIRDIDVIMGGGRKYMYPKNKTD VEYESDEKARGTRLDGLDLVDTWKSFKPR YKHSHFIWNRTELLTLDPHNVDYLLGLFEP GDMQYELNRNNVTDPSLSEMVVVAIQILR KNPKGFFLLVEGGRIDHGHHEGKAKQALH EAVEMDRAIGQAGSLTSSEDTLTVVTADHS HVFTFGGYTPRGNSIFGLAPMLSDTDKKPF TAILYGNGPGYKVVGGERENVSMVDYAH NNYQAQSAVPLRHETHGGEDVAVFSKGPM AHLLHGVHEQNYVPHVMAYAACIGANLG HCAPASSAGSLAAGPLLLALALYPLSVLF
65	人类 Osterix 蛋白	MASSLLEEEVHYGSSPLAMLTAACSKFGG SSPLRDSTTLGKAGTKKPYSVGSDLSASKT

[0744]

SEQ ID NO:	描述	序列
		MGDAYPAPFTSTNGLLSPAGSPPAPTSGYA NDYPPFSHSFPGPTGTQDPGLLVPKGHSSS DCLPSVYTSLDMTHPYGSWYKAGIHAGIS PGPGNTPTPWWDMHPGGNWLGGGQGQG DGLQGTLPTGPAQPPLNPQLPTYPSDFAPL NPAPYPAPHLLQPGPQHVLPQDVYKPKAV GNSGQLEGSGGAKPPRGASTGGSGGYGGS GAGRSSCDCPNCQELERLGAAAAGLRKKP IHSCHIPGCGKVYGKASHLKAHLRWHTGE RPFVCNWLFCGKRFTRSDELERHVRTHTR EKKFTCLLCSKRFTRSDHLSKHQRTHGEPG PGPPPSGPKELGEGRSTGEEEASQTPRPSAS PATPEKAPGGSPEQSNLLEI
66	人类 Klotho 蛋白	MPASAPPRRPRPPPPSLSLLLVLLGLGGRRL RAEPGDGAQTWARFSRPPAPEAAGLFQGT FPDGFLWAVGSAAYQTEGGWQQHGKGASI WDTFTHHPLAPPGDSRNASLPLGAPSPLQP ATGDVASDSYNNVFRDTEALRELGVTHYR FSISWARVLPNGSAGVPNREGLRYYRRLLE RLRELGVQPVVTLYHWDLPQRLQDAYGG WANRALADHFRDYAELCFRHFGGQVKYW ITIDNPYVVAWHGYATGRLAPGIRGSPRLG YLVAHNLLLAHAKVWHLYNTSFRPTQGG QVSIALSSHWINPRRMTDHSIKECQKSLDF VLGWFAKPVFIDGDYPESMKNNLSSILPDF TESEKKFIKGTADFFALCFGPTLSFQLLDPH MKFRQLESPNLRQLLSWIDLEFNHPQIFIVE NGWFVSGTTKRDDAKYMYYLKKFIMETL KAIKLDGVDVIGYTAWSLMDGFEWHRGY SIRRGLFYVDFLSQDKMLLPKSSALFYQKL IEKNGFPPLPENQPLEGTFPCDFAWGVVDN YIQVDTTLSQFTDLNVYLWDVHHSKRLIK VDGVVTKKRKSYCVDFAAIQPQIALLQEM HVTHFRFSLDWALILPLGNQSQVNHTILQY YRCMASELVRVNITPVVALWQPMAPNQGL PRLLARQGAWENPYTALAFAEYARLCFQE LGHHVKLWITMNEPYTRNMTYSAGHNLL KAHALAWHVYNEKFRHAQNGKISIALQA DWIEPACPFSQKDKEVAERVLEFDIGWLAE

[0745]

SEQ ID NO:	描述	序列
		PIFGSGDYPWVMRDWLNQRNNFLLPYFTE DEKKLIQGTFDFLALSHYTTILVDSEKEDPI KYNDYLEVQEMTDITWLNSPSQVAVVPW GLRKVLNWLKFKYGDLPMYIISNGIDDGL HAEDDQLRVYYMQNYINEALKAHILDGIN LCGYFAYSFNDRTAPRFGLYRYAADQFEPK ASMKHYRKIIDSNGFPGPETLERFCPEEFT VCTECSFFHTRKSLLAFIAFLFFASIISLSLIF YYSKKGRRSYK
67	人类 Sm22-alpha 蛋白	MANKGPSYGMSREVQSKIEKKYDEELEER LVEWIIVQCGPDVGRPDRGRLGFQVWLKN GVILSKLVNSLYPDGSKPVKVPENPPSMVF KQMEQVAQFLKAAEDYGVIKTDMFQTVD LFEGKDMAAVQRTLMALGSLAVTKNDGH YRGDPNWFMKKAQEHKREFTESQLQEGK HVIGLQMGSNRGASQAGMTGYGRPRQIIS
68	人类 alpha-SMA 蛋白	MCEEEDSTALVCDNGSGLCKAGFAGDDAP RAVFPSIVGRPRHQGVMVGMGQKDSYVG DEAQSKRGILTLKYPIEHGIITNWDDMEKI WHHSFYNELRVAPEEHPTLLTEAPLNPKAN REKMTQIMFETFNVPAMYVAIQAVLSLYAS GRTTGIVLDSGDGVTHNVPIYEGYALPHAI MRLDLAGRDLTDYLMKILTERGYSFVTTA EREIVRDIKEKLCYVALDFENEMATAASSS SLEKSYELPDGQVITIGNERFRCPETLFQPS FIGMESAGIHETTYNSIMKCDIDIRKDLYAN NVLSGGTTMYPGIADRMQKEITALAPSTM KIKIIAPPERKYSVWIGGSILASLSTFQQMW ISKQEYDEAGPSIVHRKCF
69	人类 MYOCD 蛋白	MTLLGSEHSLLIRSKFRSVLQLRLQQRRTQ EQLANQGIIPPLKRPAEFHEQRKHLDSDKA KNSLKRKARNRCNSADLVNMHILQASTAE RSIPTAQMKLKRARLADDLNEKIALRPGPL ELVEKNILPVDSAVKEAIKGNQVSFSKSTD AFAFEEDSSSDGLSPDQTRSEDPQNSAGSP PDAKASDTPSTGSLGTNQDLASGSENDRN DSASQPSHQSDAGKQGLGPPSTPIAVHAAV KSKSLGDSKNRHKKPKDPKPKVKKLKYH QYIPPDQKAEKSPPPMDSAYARLLQQQQLF

[0746]

SEQ ID NO:	描述	序列
		LQLQILSQQQQQQQHRFSYLGMHQAQLK EPNEQMVRNPNSSSTPLSNTPLSPVKNSFS GQTGVSSFKPGPLPPNLDDLKVSELRQQLR IRGLPVSGTKTALMDRLRPFQDCSGNPVPN FGDITTVTFPVTPNTLPNYQSSSSTSALSNG FYHFGSTSSSPPISPASSDLSVAGSLPDTFND ASPSFGLHPSPVHVCTEESLMSSLNGGSVP SELDGLDSEKDKMLVEKQKVINELTWKLQ QEQRQVEELRMQLQKQKRNNCSEKKPLPF LAASIKQEEAVSSCPFASQVPVKRQSSSSEC HPPACEAAQLQPLGNAHCVESSDQTNVLS STFLSPQCSPQHSPLGAVKSPQHISLPPSPN NPHFLPSSSGAQGEGHRVSSPISSQVCTAQ NSGAHDGHPPSFSPHSSSLHPPFSGAQADS SHGAGGNPCPKSPCVQQKMAGLHSSDKV GPKFSIPSPTFSKSSSAISEVTQPPSYEDAVK QQMTRSQQMDELLDVLIESGEMPADARED HSCLQKVPKIPRSSRSPTAVLTKPSASFEQA SSGSQIPFDPYATDSDEHLEVLLNSQSPLGK MSDVTLLKIGSEEPHFDGIMDGFSGKAAE DLFNAHEILPGPLSPMQTQFSPSSVDSNGL QLSFTESPWETMEWLDLTPPNSTPGFSALT TSSPSIFNIDFLDVTDLNLNSSMDLHLQQW
70	人类 alpha-SMA mRNA	CTCTCCCCGCCCCCGCGGGGCGGCGCGC ACTCACCCACCCGCGCCGGAGCGGACCT TTGGCTTGGCTTGTCAGGGCTTGTCCAG GAGTTCCGCTCCTCTCTCCAACCGGGGT CCCCCTCCAGCGACCCTAAAGCTTCCCA GACTTCCGCTTCAATTCCTGTCCGCACCC CACGCCCACCTCAACGTGGAGCGCAGTG GTCTCCGAGGAGCGCCGGAGCTGCCCCG CCTGCCCAGCGGGGTCAGCACTTCGCAT CAAGGCCCAAGAAAAGCAAGTCCTCCA GCGTTCTGAGCACCCGGGCCTGAGGGAA GGTCCTAACAGCCCCCGGGAGCCAGTCT CCAACGCCTCCCGCAGCAGCCCGCCGCT CCCAGGTGCCCGCGTGCGCCGCTGCCGC CGCAATCCCGCACGCGTCCCGCGCCCGC CCCACTTTGCCTATCCCCGGGACTAAGAC

[0747]

SEQ ID NO:	描述	序列
		GGGAATCCTGTGAAGCAGCTCCAGCTAT GTGTGAAGAAGAGGACAGCACTGCCTTG GTGTGTGACAATGGCTCTGGGCTCTGTAA GGCCGGCTTTGCTGGGGACGATGCTCCC AGGGCTGTTTTCCCATCCATTGTGGGACG TCCCAGACATCAGGGGGTGATGGTGGGA ATGGGACAAAAAGACAGCTACGTGGGTG ACGAAGCACAGAGCAAAAGAGGAATCC TGACCCTGAAGTACCCGATAGAACATGG CATCATCACCAACTGGGACGACATGGAA AAGATCTGGCACCACTCTTTCTACAATGA GCTTCGTGTTGCCCCTGAAGAGCATCCC ACCCTGCTCACGGAGGCACCCCTGAACC CCAAGGCCAACCGGGAGAAAATGACTCA AATTATGTTTGAGACTTTCAATGTCCCAG CCATGTATGTGGCTATCCAGGCGGTGCTG TCTCTCTATGCCTCTGGACGCACAACTGG CATCGTGCTGGACTCTGGAGATGGTGTC ACCCACAATGTCCCCATCTATGAGGGCTA TGCCTTGCCCCATGCCATCATGCGTCTGG ATCTGGCTGGCCGAGATCTCACTGACTAC CTCATGAAGATCCTGACTGAGCGTGGCTA TTCCTTCGTTACTACTGCTGAGCGTGAGA TTGTCCGGGACATCAAGGAGAAACTGTG TTATGTAGCTCTGGACTTTGAAAATGAGA TGGCCACTGCCGCATCCTCATCCTCCCTT GAGAAGAGTTACGAGTTGCCTGATGGGC AAGTGATCACCATCGGAAATGAACGTTT CCGCTGCCCAGAGACCCTGTTCCAGCCA TCCTTCATCGGGATGGAGTCTGCTGGCAT CCATGAAACCACCTACAACAGCATCATG AAGTGTGATATTGACATCAGGAAGGACC TCTATGCTAACAATGTCCTATCAGGGGGC ACCACTATGTACCCTGGCATTGCCGACCG AATGCAGAAGGAGATCACGGCCCTAGCA CCCAGCACCATGAAGATCAAGATCATTGC CCCTCCGGAGCGCAAATACTCTGTCTGGA TCGGTGGCTCCATCCTGGCCTCTCTGTCC ACCTTCCAGCAGATGTGGATCAGCAAAC AGGAATACGATGAAGCCGGGCCTTCCATT

[0748]

SEQ ID NO:	描述	序列
		GTCCACCGCAAATGCTTCTAAAACACTTT CCTGCTCCTCTCTGTCTCTAGCACACAAC TGTGAATGTCCTGTGGAATTATGCCTTCA GTTCTTTTCCAAATCATTCCTAGCCAAAG CTCTGACTCGTTACCTATGTGTTTTTTAAT AAATCTGAAATAGGCTACTGGTAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAA
71	人类 MYOCD mRNA	AATCGCCGGCAGCCTATGACATCAGACA GGAACGCCTGGGATGCCGCGCTGCTCCT GGCCAACCTCCGAGGAGGAGGAGGGTC CCGCCGGCTAAGAGTTAATTAGCCCCGC ACGGCGAGGGGGGAGGCGCCAGTTTTCT GGGGACACTGGCTGCCACTGTACTCCTA CCCAGGGGAGCTCACGGAGAGTTGGATG AATTCTGGGTTGTTAGCTGCGGTCAGCTG GGCTCCCGGGAGCCTGTTGCTGGTGGAG AACAGGGGGCGCCTGGCCAAGGGACCA GCGGCTTGCTGAGACTCAACATGACACT CCTGGGGTCTGAGCATTCCTTGCTGATTA GGAGCAAGTTCAGATCAGTTTTACAGTTA AGACTTCAACAAAGAAGGACCCAGGAA CAACTGGCTAACCAAGGCATAATACCAC CACTGAAACGTCCAGCTGAATTCCATGA GCAAAGAAAACATTTGGATAGTGACAAG GCTAAAAATTCCCTGAAGCGCAAAGCCA GAAACAGGTGCAACAGTGCCGACTTGGT TAATATGCACATACTCCAAGCTTCCACTG CAGAGAGGTCCATTCCAACTGCTCAGAT GAAGCTGAAAAGAGCCCGACTCGCCGAT GATCTCAATGAAAAAATTGCTCTACGACC AGGGCCACTGGAGCTGGTGGAAAAAAA CATTCTTCCTGTGGATTCTGCTGTGAAAG AGGCCATAAAAGGTAACCAGGTGAGTTT CTCCAAATCCACGGATGCTTTTGCCTTTG AAGAGGACAGCAGCAGCGATGGGCTTTC TCCGGATCAGACTCGAAGTGAAGACCCC CAAAACTCAGCGGGATCCCCGCCAGACG CTAAAGCCTCAGATACCCCTTCGACAGGT

[0749]

SEQ ID NO:	描述	序列
		TCTCTGGGGACAAACCAGGATCTTGCTT CTGGCTCAGAAAATGACAGAAATGACTC AGCCTCACAGCCCAGCCACCAGTCAGAT GCGGGGAAGCAGGGGCTTGGCCCCCCCA GCACCCCCATAGCCGTGCATGCTGCTGTA AAGTCCAAATCCTTGGGTGACAGTAAGA ACCGCCACAAAAAGCCCAAGGACCCCA AGCCAAAGGTGAAGAAGCTTAAATATCA CCAGTACATTCCCCCAGACCAGAAGGCA GAGAAGTCCCCTCCACCTATGGACTCAG CCTACGCTCGGCTGCTCCAGCAACAGCA GCTGTTCCTGCAGCTCCAAATCCTCAGCC AGCAGCAGCAGCAGCAGCAACACCGATT CAGCTACCTAGGGATGCACCAAGCTCAG CTTAAGGAACCAAATGAACAGATGGTCA GAAATCCAAACTCTTCTTCAACGCCACT GAGCAATACCCCCTTGTCTCCTGTCAAAA ACAGTTTTTCTGGACAAACTGGTGTCTCT TCTTTCAAACCAGGCCCACTCCCACCTAA CCTGGATGATCTGAAGGTCTCTGAATTAA GACAACAGCTTCGAATTCGGGGCTTGCC TGTGTCAGGCACCAAAACGGCTCTCATG GACCGGCTTCGACCCTTCCAGGACTGCT CTGGCAACCCAGTGCCGAACTTTGGGGA TATAACGACTGTCACTTTTCCTGTCACAC CCAACACGCTGCCCAATTACCAGTCTTCC TCTTCTACCAGTGCCCTGTCCAACGGCTT CTACCACTTTGGCAGCACCAGCTCCAGC CCCCCGATCTCCCCAGCCTCCTCTGACCT GTCAGTCGCTGGGTCCCTGCCGGACACC TTCAATGATGCCTCCCCCTCCTTCGGCCT GCACCCGTCCCCAGTCCACGTGTGCACG GAGGAAAGTCTCATGAGCAGCCTGAATG GGGGCTCTGTTCCTTCTGAGCTGGATGG GCTGGACTCCGAGAAGGACAAGATGCTG GTGGAGAAGCAGAAGGTGATCAATGAAC TCACCTGGAAACTCCAGCAAGAGCAGAG GCAGGTGGAGGAGCTGAGGATGCAGCTT CAGAAGCAGAAAAGGAATAACTGTTCAG AGAAGAAGCCGCTGCCTTTCCTGGCTGC

[0750]

SEQ ID NO:	描述	序列
		CTCCATCAAGCAGGAAGAGGCTGTCTCC AGCTGTCCTTTTGCATCCCAAGTACCTGT GAAAAGACAAAGCAGCAGCTCAGAGTG TCACCCACCGGCTTGTGAAGCTGCTCAA CTCCAGCCTCTTGGAAATGCTCATTGTGT GGAGTCCTCAGATCAAACCAATGTACTTT CTTCCACATTTCTCAGCCCCCAGTGTTCC CCTCAGCATTCACCGCTGGGGGCTGTGA AAAGCCCACAGCACATCAGTTTGCCCCC ATCACCCAACAACCCTCACTTTCTGCCCT CATCCTCCGGGGCCCAGGGAGAAGGGCA CAGGGTCTCCTCGCCCATCAGCAGCCAG GTGTGCACTGCACAGAACTCAGGAGCAC ACGATGGCCATCCTCCAAGCTTCTCTCCC CATTCTTCCAGCCTCCACCCGCCCTTCTC TGGAGCCCAAGCAGACAGCAGTCATGGT GCCGGGGGGAAACCCTTGTCCCAAAAGCC CATGTGTACAGCAAAAGATGGCTGGTTTA CACTCTTCTGATAAGGTGGGGCCAAAGT TTTCAATTCCATCCCCAACTTTTTCTAAGT CAAGTTCAGCAATTTCAGAGGTAACACA GCCTCCATCCTATGAAGATGCCGTAAAGC AGCAAATGACCCGGAGTCAGCAGATGGA TGAACTCCTGGACGTGCTTATTGAAAGC GGAGAAATGCCAGCAGACGCTAGAGAG GATCACTCATGTCTTCAAAAAGTCCCAA AGATACCCAGATCTTCCCGAAGTCCAACT GCTGTCCTCACCAAGCCCTCGGCTTCCTT TGAACAAGCCTCTTCAGGCAGCCAGATC CCCTTTGATCCCTATGCCACCGACAGTGA TGAGCATCTTGAAGTCTTATTAAATTCCC AGAGCCCCCTAGGAAAGATGAGTGATGT CACCCTTCTAAAAATTGGGAGCGAAGAG CCTCACTTTGATGGGATAATGGATGGATT CTCTGGGAAGGCTGCAGAAGACCTCTTC AATGCACATGAGATCTTGCCAGGCCCCCT CTCTCCAATGCAGACACAGTTTTCACCCT CTTCTGTGGACAGCAATGGGCTGCAGTT AAGCTTCACTGAATCTCCCTGGGAAACC ATGGAGTGGCTGGACCTCACTCCGCCAA

[0751]

SEQ ID NO:	描述	序列
		ATTCCACACCAGGCTTTAGCGCCCTCACC ACCAGCAGCCCCAGCATCTTCAACATCG ATTTCCTGGATGTCACTGATCTCAATTTG AATTCTTCCATGGACCTTCACTTGCAGCA GTGGTAGAATGCCCAATGCACCAGTGCTA TGGAAGACCAATGGAGTTCCATGGGGGA AAGCACACAGCCATACATACTTTACTGTC CAAAAACAGAAGAAGAAGAAGAGAATT AAAAAGAAGCAATGATTTCTGTGCCAAT GAACAAGAACAAAAGTCATTTTTAGAAA TACATATACTGTAATATTTACCAACAGTCA GTAACTGTTAATGATTTCAACAATGCATT AAAAGAATGTGCTTTCTCAGATTAAGGAT GCCAAAAAAAGATATTTCACTGCCTTTTCA AAGACCAGTATATTTTCTAGCCCATAATTT TTCTCAGGCATTGTTGGGGCATAAGCTCA CACTGTAAGCTTTTCTCATGAATTCACTA GACATAACGTGGAAGGAAAACGTAGTCT TTTGGGAGTACAGGGAAGCCAGCCCCTC AAAGCTTATGGAAGACATACCTGCAATG GAAGCTGTTGCCCAATGTCTCCATTACTA TCTTTCAAAAGAGAAGCCAGACCCAGCT TCAGATCAAAAGTTCTTGAGACAGAGGA ACAAAACCAATCGATTTCCAGGGAAGCT AATCAACTCTCTTTTCCCTCTACCACAAA ACTGCCCTGCTGGAGTGGTTCTGAACCT GTACCCAGGACTCGATGTGGTCACTAATA ACAATTAACCTGAACTGAGTCCACAGAA CTCCACTCGGAACTTTCTTCTTTTTTAACT AGTGGCCCAATCATTCCCACCATCTCTGT GCTGATAAGTACGTGTCCTAGATGAGAAC CCTGAAGAATGCAGACCTTCTTCCCCCG AAGGAGATGCCACAAGCTCTCCAACACA GCCCCCTTTAGTTCCAAAGACTAGAGATG ACCACATTGGTAGAAGTATATCTCGAGGC ACAGGAAGGGAGCCCCACCAGGGATAAT TCAGACAGGACTAGAGAATAACATCATTT CACATACCCTGGGATAAACACCCTGGGTT CCTATAGAAGGACTATTACTTATGGGAGT CCAACTTCTCCTTTTGTTTTGTTATTATCA

[0752]

SEQ ID NO:	描述	序列
		GTTTATCTTTCTCCCACTCCACTTTTCCTT CAAGGTACCAATCCTTTCCTGTTCCTCGT TTGGCCATCTTTCTTTTTCTGCCTCCACAT TGGGAGGGGAGGACTTCTCAGTTCTAAC AAGCTGCCATACTCCTAAGAAAGCCATTT TTGAAAAATTTAACAATCCAGGTTCTTCT GGAGAACTCATTCTCCACACGCACAGTT TGCTGCAAAAGGAAGTTGCAAGAATTTC TTGAGGAAGAAACTGGTGACTTGGTCCA TCAGTCACGAAGTTCTTTCTATTCTCGTTT AGTTTTCAAGAAATTATTGGTTTGTGTTG CTCTGGGGAAATTGGAAATCATTACATTG TAAAGACAAATATGGATGATATTTACAAG AGAGAATTTCAGATCTGGGTTTTTGAAA GAAAACAGAATTGCGCATTGAAAACGAT GGAAGGAAAAAGACAATGGTCTAATGTG CATTCCTCATTACCTCTCGTGGCTTTGGCT GGGAGTTGGAAAAAGCTAAAATTTCAGA ACAGTCTCTGTAAGGCTCTCTGTGGCTCC AGTTCACCATTTTATATTGTTGCATGCTGT AGAAAGGAGCTATTGCTGTTGTTTTGTTT TTTTATTTAAATCACTAAGGCACTGTTTTT ATCTTTTGTAAAAAAAAAAAAAAAGTTG TTCACTGTGCACTTATAGAAAAAATAATC AAAAATGTTGGGATTTTAGAAGCTCTCTT TTTGATAAACCAAAGATTTAGAAGTCATT CCATTGTTAACTTGTAAAAATGTGTGAAC ACAGAGAGTTTTTGGTGATTGCTACTCTG AAAGCTGCCAGATCTTATTCTGGGGGTGG GATGTGGAGGAATACACATACACACACA AACATACATGTATGTATAATAGATATATAC ATATGTGTATATTATATCTGTGTGTGCATGT ATCTCCAAAAGCGGCGTTACAGAGTTCT ACACCAAAAGCCTTTAACCCTTAATCTGC TGTGAATGATACCTGGCCTTTCTCACTAT GAATTTCTGATTAACCAACCAGACTACAC GTTGCCTCTCTGTGTATGACTAACGGCTC CAACCCGATGACTCACAGCTACTTGCTTA TCGTGAACAAGCTCATCTTGGCAATGAAT ATGGATGTGAAAAGACAGAACAGCTTCA

[0753]

SEQ ID NO:	描述	序列
		CCATTAGTAGCTGGAAATGGTATCACAGT CTCTTATAGAGGAATATGAAAGGAACAA GAAAATCATTTTACATTCCTTTTATCTGTA TTGTGCTTTAAAAGATCCACATGGTAAAT TTTTTATTTTGCTTTTATGTCAGTCATCAG AACCAAAAAAATCCAGAAGAAAAAATTG CCAGTGTTTCCTTTGAAGATGAAGCTACT GGGGAAGAAAACCTTATTAATACACTCC ACACATTTGTTCATTCCTCAGCTGTTGGT GTTTTCTTGGGGTCTTGACAAAGCTTGCT GGTCAGTGCACTTTTCAGGTGTCACGTTT TGCTGTTTGTATGTTTTTTCTTCCCCTTAC TTCCTTTGGAAAACAAACTCACACAGTG CCCCTACTCTGAGACCTGGGACTGAGTG TTAATTATTTTTTCCTTGGGTATTTCTATCT GAGAGACTAGACCTAGTTAGGAGGCCTC TGTACTTCTCCAGATTGTACCTTTTTATGG GGATCTTTGAGGCTATGACCCAGGACTGA TAGATATGCCTTACGGAAGACAAAAGATA AAATGGTTCCTATATCCTAATGCAAACCA ACACAGTTAAAAGAGCAGATCTCTGGAT AACTGCTCTCAACCTGCTTCTACAGTCTC CACAAACCGCATTCACCCTCTCTCTTCAT AGCTCAGACATGAAATTTGAGGGAGAAA ACTGGAGATAATTGGGAGAAAATTGATG AAGTTGGCTGCTTCCAGTAGATCAGATAA TCCATGAATTTGTCTCCCATTGAGAATTTT ATTTTAAATTCTTTTAAACTCTTCGTTGTG TCTTTTGTGATGACAAATCAGGCATGACT AAAAGATGTACAGAGACTTACGAAGATG GTCACATTCAAGTTCCCTAATGCTCTTAG AACCTGAAGATGACCATGTGTAGTTTTCT TAAGACCTCTGAACCCCCATGGTGATGA AGACTTGAAGACATTTGCAGCTATCTGCT GCAGTCTGGTAGATTCATACTTATCTAAA GAAGTCAAAAAATTTATTCGTGCAAGTG CTTGCAGGAAGCCAGTGCTTATTAGTAGT GACCCTGCTTCTATCAACGTTATTGAGAC AACACATATTCTATTCTAAGGGAGAAAGA GGGAGGAAGAGAGGGAGGGAGGGAGG

[0754]

SEQ ID NO:	描述	序列
		AAGAAGAGGGAGGGAGCGAGGAAGGAA GATAGGAGATGGGTAGGGGGGTAAAGAG AAAAGGAGGGAGAAGGGAAGGAAGGA AAGAAGAGAGGAAAGAAAGGAGGGAA GGAAAAAAGGGCCAAACTTTCTGATCTA TGAACTTCTCAGTTCAGCTGTCACATTAT GAGAAGTAAATCAGAATTTTTTTAAGGA GAAGTCATTCTTAGCACTACAATAATTGT ACCAGTAATTGAGGAAACCAAGACAATC TTCACCTGAATAATAGAGGGTCTGAGAA CTGTCAGCCTTTTGCCATTCAAAAACATT TATGTCCAACCTGAAAAAAAAAGCATCAAT AAAACCTATCCCAAGCATTCAAAATAGTC CTTTCCAAATGTTATTTATTTTAAAGTCAA TCAGCTCTTTTAGAAACAGATTCTGGTCT GGCTGAAAACTCCCACAACAAATTTACT CATCCAGTGGCTAATATTTAATGCCCACC ATGGGCAGAGCACACAAATCTTCAGATA AACAATACTGAATTGATTCAGCACAGATT GTTCAGATTTGAATGACCAGGGAGTTGTA TTTGCACATGCAAGAACACTAAGAATCTC CCAGTCCTCAAACTAGAAACCTTTCAGCT ACGATGAAAAAAAAAAAGGGGTCTTCAT TTTTCCAAGAGGGGGTGGAGGTGGGGAT CACTTTTTAGCTAAAAGCTATCTCTCACT TCAAAATTCTTGTCTTTTTCTTTGTGGAC AAACACCAGTAGTCTATCACTTGGAGATC TTTTAATATCTCCCATCATTTAAAACATCC ACGAGAGTTTGAAGATTTGTGTTGATTGC CAGATACAGAAGCCCCTTGAAAATAAGG AAAGGGTGGAGGAAGCATTTTTGTGTCC TATCCCTACTTATCGTAGCAGCTCTATAGA CAAAAGGGACACTTACTGGTGAGCCTCT GGCCCTTAAAAGAAAATCATCTAAGAATA TGAAGGCAATTTGATTTCCCCCCACAGCC CTCAGCTGCCTTCCTCACAGAAGGAAGT TCCCAAAATTGCTGGTACACAGTTTGCAA TCAAATATCAGATATGAGAAAACCTGTAG TGAAGAGTCTGGGTTCTTGGTTTTCTCAT AAATCCAATATAAATTTGTAGGTTGGTTC

[0755]

SEQ ID NO:	描述	序列
		AGGGTCAAAATTGCCAGTGCTTTATTAGA CAGATGATACTGATAGACACACAGAGCC CAGGTCCTGGAACAAGACAATCCTGTAG TGCCAAGATCTGGTCAGTTGCGTTAAGG AGCTGGGTTTGATTCTAGAGTCCAGGTTT ATAGAGAAACCCTGGCTAGATTGAGCCT ACCCATGGGGAGACGATTTCAAGACAGG ATGAGATCTGGGAAGAATTTTGTTGTCAT CTGCCAGGGAAATTATCACAGGACTCATT GAATGCAATAACATGTGAGTAAGTTCCCT TTTGATTCTGGGAATCAGCGATTTTCCCT GTGGATTAAGACAAACCAACGCCAGAAG GTCTCCTGTGCTTATTTTAACCATCTGCTC CCATCGTGAACCCTGGAGCATGCATTTCC TAGAAGTGGTTTCATAGCTCCTGTGTGTT CATGGAAAAGGGGAGTATAATGATGGGG ATGCTGGAAGCTTTTTTAATGTTTTCCAA AGGAAAGGAACCCACACTGCTCCCCAGA GTTCCTTTCCAATGGCCCTGCAGTAAGAA CGGAGGACAATGTATTGCTGGGTGCTTAA AATCCTCCCTCAGTGAAGCACAAAGAGA CACTTTGTAAAGAAAAAAAGAGCAAGCA TAGGTTCTCTGTGGGACCTTGTGGAGTG GTGTTTTCACGTTGGTCTCTTTGGCTCAA TTGAGCATAATCAGAAAGAAATGTGGGTT ATTGGGAAGAGACAAAAAGCAGTGGCTA AAATACCAAAGTTGGCATGTGTTCTTTTT TAAAAAAAAAAAAAAATGCATATATTTTT AAATAAAATGTTTATTTTAAAAAGAAAAA AAAAAAAAAAAAA
72	人类 BSAP 蛋白	MISPFLVLAIGTCLTNSLVPEKEKDPKYWR DQAQETLKYALELQKLNTNVAKNVIMFLG DGMGVSTVTAARILKGQLHHNPGEETRLE MDKFPFVALSKTYNTNAQVPDSAGTATAY LCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSA AYAHSADRDWYSDNEMPPEALSQGCKDIA YQLMHNIRDIDVIMGGGRKYMYPKNKTD VEYESDEKARGTRLDGLDLVDTWKSFKPR

[0756]

SEQ ID NO:	描述	序列
		YKHSHFIWNRTELLTLDPHNVDYLLGLFEP GDMQYELNRNNVTDPSLSEMVVVAIQILR KNPKGFFLLVEGGRIDHGHHEGKAKQALH EAVEMDRAIGQAGSLTSSEDTLTVVTADHS HVFTFGGYTPRGNSIFGLAPMLSDTDKKPF TAILYGNGPGYKVVGGERENVSMVDYAH NNYQAQSAVPLRHETHGGEDVAVFSKGPM AHLLHGVHEQNYVPHVMAYAACIGANLG HCAPASSAGSLAAGPLLLALALYPLSVLF
73	人类 BSAP mRNA	CCGGGCCTCACTCGGGCCCCGCGGCCGC CTTTATAAGGCGGCGGGGGTGGTGGCCC GGGCCGCGTTGCGCTCCCGCCACTCCGC GCCCGCTATCCTGGCTCCGTGCTCCCACG CGCTTGTGCCTGGACGGACCCTCGCCAG TGCTCTGCGCAGGATTGGAACATCAGTTA ACATCTGACCACTGCCAGCCCACCCCCT CCCACCCACGTCGATTGCATCTCTGGGCT CCAGGGATAAAGCAGGTCTTGGGGTGCA CCATGATTTCACCATTCTTAGTACTGGCCA TTGGCACCTGCCTTACTAACTCCTTAGTG CCAGAGAAAGAGAAAGACCCCAAGTAC TGGCGAGACCAAGCGCAAGAGACACTG AAATATGCCCTGGAGCTTCAGAAGCTCA ACACCAACGTGGCTAAGAATGTCATCATG TTCCTGGGAGATGGGATGGGTGTCTCCA CAGTGACGGCTGCCCGCATCCTCAAGGG TCAGCTCCACCACAACCCTGGGGAGGAG ACCAGGCTGGAGATGGACAAGTTCCCCT TCGTGGCCCTCTCCAAGACGTACAACAC CAATGCCCAGGTCCCTGACAGCGCCGGC ACCGCCACCGCCTACCTGTGTGGGGTGA AGGCCAATGAGGGCACCGTGGGGGTAAG CGCAGCCACTGAGCGTTCCCGGTGCAAC ACCACCCAGGGGAACGAGGTCACCTCCA TCCTGCGCTGGGCCAAGGACGCTGGGAA ATCTGTGGGCATTGTGACCACCACGAGA GTGAACCATGCCACCCCCAGCGCCGCCT ACGCCCACTCGGCTGACCGGGACTGGTA CTCAGACAACGAGATGCCCCCTGAGGCC

[0757]

SEQ ID NO:	描述	序列
		TTGAGCCAGGGCTGTAAGGACATCGCCT ACCAGCTCATGCATAACATCAGGGACATT GACGTGATCATGGGGGGTGGCCGGAAAT ACATGTACCCCAAGAATAAAACTGATGTG GAGTATGAGAGTGACGAGAAAGCCAGG GGCACGAGGCTGGACGGCCTGGACCTCG TTGACACCTGGAAGAGCTTCAAACCGAG ATACAAGCACTCCCACTTCATCTGGAACC GCACGGAACTCCTGACCCTTGACCCCCA CAATGTGGACTACCTATTGGGTCTCTTCG AGCCAGGGGACATGCAGTACGAGCTGAA CAGGAACAACGTGACGGACCCGTCACTC TCCGAGATGGTGGTGGTGGCCATCCAGA TCCTGCGGAAGAACCCCAAAGGCTTCTT CTTGCTGGTGGAAGGAGGCAGAATTGAC CACGGGCACCATGAAGGAAAAGCCAAG CAGGCCCTGCATGAGGCGGTGGAGATGG ACCGGGCCATCGGGCAGGCAGGCAGCTT GACCTCCTCGGAAGACACTCTGACCGTG GTCACTGCGGACCATTCCCACGTCTTCAC ATTTGGTGGATACACCCCCCGTGGCAACT CTATCTTTGGTCTGGCCCCCATGCTGAGT GACACAGACAAGAAGCCCTTCACTGCCA TCCTGTATGGCAATGGGCCTGGCTACAAG GTGGTGGGCGGTGAACGAGAGAATGTCT CCATGGTGGACTATGCTCACAACAACTAC CAGGCGCAGTCTGCTGTGCCCCTGCGCC ACGAGACCCACGGCGGGGAGGACGTGG CCGTCTTCTCCAAGGGCCCCATGGCGCA CCTGCTGCACGGCGTCCACGAGCAGAAC TACGTCCCCCACGTGATGGCGTATGCAGC CTGCATCGGGGCCAACCTCGGCCACTGT GCTCCTGCCAGCTCGGCAGGCAGCCTTG CTGCAGGCCCCCTGCTGCTCGCGCTGGC CCTCTACCCCCTGAGCGTCCTGTTCTGAG GGCCCAGGGCCCGGGCACCCACAAGCCC GTGACAGATGCCAACTTCCCACACGGCA GCCCCCCCCCTCAAGGGGCAGGGAGGTGG GGGCCTCCTCAGCCTCTGCAACTGCAAG AAAGGGGACCCAAGAAACCAAAGTCTG

[0758]

SEQ ID NO:	描述	序列
		CCGCCCACCTCGCTCCCCTCTGGAATCTT CCCCAAGGGCCAAACCCACTTCTGGCCT CCAGCCTTTGCTCCCTCCCCGCTGCCCTT TGGCCAACAGGGTAGATTTCTCTTGGGC AGGCAGAGAGTACAGACTGCAGACATTC TCAAAGCCTCTTATTTTTCTAGCGAACGT ATTTCTCCAGACCCAGAGGCCCTGAAGC CTCCGTGGAACATTCTGGATCTGACCCTC CCAGTCTCATCTCCTGACCCTCCCACTCC CATCTCCTTACCTCTGGAACCCCCCAGGC CCTACAATGCTCATGTCCCTGTCCCCAGG CCCAGCCCTCCTTCAGGGGAGTTGAGGT CTTTCTCCTCAGGACAAGGCCTTGCTCAC TCACTCACTCCAAGACCACCAGGGTCCC AGGAAGCCGGTGCCTGGGTGGCCATCCT ACCCAGCGTGGCCCAGGCCGGGAAGAG CCACCTGGCAGGGCTCACACTCCTGGGC TCTGAACACACACGCCAGCTCCTCTCTG AAGCGACTCTCCTGTTTGGAACGGCAAA AAAAAATTTTTTTTCTCTTTTTGGTGGT GGTTAAAAGGGAACACAAAACATTTAAA TAAAACTTTCCAAATATTTCCGAGGACAA AAAAAAAAA
74	Snai1 mRNA	ATTCATTGCGCCGCGGCACGGCCTAGCG AGTGGTTCTTCTGCGCTACTGCTGCGCGA ATCGGCGACCCCAGTGCCTCGACCACTAT GCCGCGCTCTTTCCTCGTCAGGAAGCCC TCCGACCCCAATCGGAAGCCTAACTACA GCGAGCTGCAGGACTCTAATCCAGAGTT TACCTTCCAGCAGCCCTACGACCAGGCC CACCTGCTGGCAGCCATCCCACCTCCGG AGATCCTCAACCCCACCGCCTCGCTGCC AATGCTCATCTGGGACTCTGTCCTGGCGC CCCAAGCCCAGCCAATTGCCTGGGCCTC CCTTCGGCTCCAGGAGAGTCCCAGGGTG GCAGAGCTGACCTCCCTGTCAGATGAGG ACAGTGGGAAAGGCTCCCAGCCCCCCAG CCCACCCTCACCGGCTCCTTCGTCCTTCT CCTCTACTTCAGTCTCTTCCTTGGAGGCC

[0759]

SEQ ID NO:	描述	序列
		GAGGCCTATGCTGCCTTCCCAGGCTTGGG CCAAGTGCCCAAGCAGCTGGCCCAGCTC TCTGAGGCCAAGGATCTCCAGGCTCGAA AGGCCTTCAACTGCAAATACTGCAACAA GGAATACCTCAGCCTGGGTGCCCTCAAG ATGCACATCCGAAGCCACACGCTGCCCT GCGTCTGCGGAACCTGCGGGAAGGCCTT CTCTAGGCCCTGGCTGCTACAAGGCCATG TCCGGACCCACACTGGCGAGAAGCCCTT CTCCTGTCCCCACTGCAGCCGTGCCTTCG CTGACCGCTCCAACCTGCGGGCCCACCT CCAGACCCACTCAGATGTCAAGAAGTAC CAGTGCCAGGCGTGTGCTCGGACCTTCT CCCGAATGTCCCTGCTCCACAAGCACCA AGAGTCCGGCTGCTCAGGATGTCCCCGC TGACCCTCGAGGCTCCCTCTTCCTCTCCA TACCTGCCCCTGCCTGACAGCCTTCCCCA GCTCCAGCAGGAAGGACCCCACATCCTT CTCACTGCCATGGAATTCCCTCCTGAGTG CCCCACTTCTGGCCACATCAGCCCCACA GGACTTTGATGAAGACCATTTTCTGGTTC TGTGTCCTCTGCCTGGGCTCTGGAAGAG GCCTTCCCATGGCCATTTCTGTGGAGGGA GGGCAGCTGGCCCCCAGCCCTGGGGGAT TCCTGAGCTGGCCTGTCTGCGTGGGTTTT TGTATCCAGAGCTGTTTGGATACAGCTGC TTTGAGCTACAGGACAAAGGCTGACAGA CTCACTGGGAAGCTCCCACCCCACTCAG GGGACCCCACTCCCCTCACACACACCCC CCCACAAGGAACCCTCAGGCCACCCTCC ACGAGGTGTGACTAACTATGCAATAATCC ACCCCCAGGTGCAGCCCCAGGGCCTGCG GAGGCGGTGGCAGACTAGAGTCTGAGAT GCCCCGAGCCCAGGCAGCTATTTCAGCC TCCTGTTTGGTGGGGTGGCACCTGTTTCC CGGGCAATTTAACAATGTCTGAAAAGGG ACTGTGAGTAATGGCTGTCACTTGTCGGG GGCCCAAGTGGGGTGCTCTGGTCTGACC GATGTGTCTCCCAGAACTATTCTGGGGGC CCGACAGGTGGGCCTGGGAGGAAGATGT

[0760]

SEQ ID NO:	描述	序列
		TTACATTTTTAAAGGTACACTGGTATTTAT ATTTCAAACATTTTGTATCAAGGAAACGT TTTGTATAGTTATATGTACAGTTTATTGATA TTCAATAAAGCAGTTAATTTATATATTAAA AAAAAAAAAAAAAAA
75	Dkk1 mRNA	GCAGAGCTCTGTGCTCCCTGCAGTCAGG ACTCTGGGACCGCAGGGGGCTCCCGGAC CCTGACTCTGCAGCCGAACCGGCACGGT TTCGTGGGGACCCAGGCTTGCAAAGTGA CGGTCATTTTCTCTTTCTTTCTCCCTCTTG AGTCCTTCTGAGATGATGGCTCTGGGCGC AGCGGGAGCTACCCGGGTCTTTGTCGCG ATGGTAGCGGCGGCTCTCGGCGGCCACC CTCTGCTGGGAGTGAGCGCCACCTTGAA CTCGGTTCTCAATTCCAACGCTATCAAGA ACCTGCCCCCACCGCTGGGCGGCGCTGC GGGGCACCCAGGCTCTGCAGTCAGCGCC GCGCCGGGAATCCTGTACCCGGGCGGGA ATAAGTACCAGACCATTGACAACTACCA GCCGTACCCGTGCGCAGAGGACGAGGAG TGCGGCACTGATGAGTACTGCGCTAGTCC CACCCGCGGAGGGGACGCAGGCGTGCA AATCTGTCTCGCCTGCAGGAAGCGCCGA AAACGCTGCATGCGTCACGCTATGTGCTG CCCCGGGAATTACTGCAAAAATGGAATAT GTGTGTCTTCTGATCAAAATCATTTCCGA GGAGAAATTGAGGAAACCATCACTGAAA GCTTTGGTAATGATCATAGCACCTTGGAT GGGTATTCCAGAAGAACCACCTTGTCTTC AAAAATGTATCACACCAAAGGACAAGAA GGTTCTGTTTGTCTCCGGTCATCAGACTG TGCCTCAGGATTGTGTTGTGCTAGACACT TCTGGTCCAAGATCTGTAAACCTGTCCTG AAAGAAGGTCAAGTGTGTACCAAGCATA GGAGAAAAGGCTCTCATGGACTAGAAAT ATTCCAGCGTTGTTACTGTGGAGAAGGTC TGTCTTGCCGGATACAGAAAGATCACCAT CAAGCCAGTAATTCTTCTAGGCTTCACAC TTGTCAGAGACACTAAACCAGCTATCCA

[0761]

SEQ ID NO:	描述	序列
		AATGCAGTGAACTCCTTTTATATAATAGAT GCTATGAAAACCTTTTATGACCTTCATCA ACTCAATCCTAAGGATATACAAGTTCTGT GGTTTCAGTTAAGCATTCCAATAACACCT TCCAAAAACCTGGAGTGTAAGAGCTTTG TTTCTTTATGGAACTCCCCTGTGATTGCA GTAAATTACTGTATTGTAAATTCTCAGTGT GGCACTTACCTGTAAATGCAATGAAACTT TTAATTATTTTTCTAAAGGTGCTGCACTG CCTATTTTTCCTCTTGTTATGTAAATTTTT GTACACATTGATTGTTATCTTGACTGACA AATATTCTATATTGAACTGAAGTAAATCAT TTCAGCTTATAGTTCTTAAAAGCATAACC CTTTACCCCATTTAATTCTAGAGTCTAGA ACGCAAGGATCTCTTGGAATGACAAATG ATAGGTACCTAAAATGTAACATGAAAATA CTAGCTTATTTTCTGAAATGTACTATCTTA ATGCTTAAATTATATTTCCCTTTAGGCTGT GATAGTTTTTGAAATAAAATTTAACATTTA ATATCATGAAATGTTATAAGTAGACATACA TTTTGGGATTGTGATCTTAGAGGTTTGTG TGTGTGTACGTATGTGTGTGTTCTACAAG AACGGAAGTGTGATATGTTTAAAGATGAT CAGAGAAAAGACAGTGTCTAAATATAAG ACAATATTGATCAGCTCTAGAATAACTTT AAAGAAAGACGTGTTCTGCATTGATAAA CTCAAATGATCATGGCAGAATGAGAGTG AATCTTACATTACTACTTTCAAAAATAGTT TCCAATAAATTAATAATACCTAAAAAAAA AAA
76	Col1a1 mRNA	TCGTCGGAGCAGACGGGAGTTTCTCCTC GGGGTCGGAGCAGGAGGCACGCGGAGT GTGAGGCCACGCATGAGCGGACGCTAAC CCCCTCCCCAGCCACAAAGAGTCTACAT GTCTAGGGTCTAGACATGTTCAGCTTTGT GGACCTCCGGCTCCTGCTCCTCTTAGCGG CCACCGCCCTCCTGACGCACGGCCAAGA GGAAGGCCAAGTCGAGGGCCAAGACGA AGACATCCCACCAATCACCTGCGTACAG

[0762]

SEQ ID NO:	描述	序列
		AACGGCCTCAGGTACCATGACCGAGACG TGTGGAAACCCGAGCCCTGCCGGATCTG CGTCTGCGACAACGGCAAGGTGTTGTGC GATGACGTGATCTGTGACGAGACCAAGA ACTGCCCCGGCGCCGAAGTCCCCGAGGG CGAGTGCTGTCCCGTCTGCCCCGACGGC TCAGAGTCACCCACCGACCAAGAAACCA CCGGCGTCGAGGGACCCAAGGGAGACA CTGGCCCCCGAGGCCCAAGGGGACCCGC AGGCCCCCCTGGCCGAGATGGCATCCCT GGACAGCCTGGACTTCCCGGACCCCCCG GACCCCCCGGACCTCCCGGACCCCCTGG CCTCGGAGGAAACTTTGCTCCCCAGCTG TCTTATGGCTATGATGAGAAATCAACCGG AGGAATTTCCGTGCCTGGCCCCATGGGTC CCTCTGGTCCTCGTGGTCTCCCTGGCCCC CCTGGTGCACCTGGTCCCCAAGGCTTCC AAGGTCCCCCTGGTGAGCCTGGCGAGCC TGGAGCTTCAGGTCCCATGGGTCCCCGA GGTCCCCCAGGTCCCCCTGGAAAGAATG GAGATGATGGGGAAGCTGGAAAACCTGG TCGTCCTGGTGAGCGTGGGCCTCCTGGG CCTCAGGGTGCTCGAGGATTGCCCGGAA CAGCTGGCCTCCCTGGAATGAAGGGACA CAGAGGTTTCAGTGGTTTGGATGGTGCC AAGGGAGATGCTGGTCCTGCTGGTCCTA AGGGTGAGCCTGGCAGCCCTGGTGAAAA TGGAGCTCCTGGTCAGATGGGCCCCCGT GGCCTGCCTGGTGAGAGAGGTCGCCCTG GAGCCCCTGGCCCTGCTGGTGCTCGTGG AAATGATGGTGCTACTGGTGCTGCCGGG CCCCCTGGTCCCACCGGCCCCGCTGGTC CTCCTGGCTTCCCTGGTGCTGTTGGTGCT AAGGGTGAAGCTGGTCCCCAAGGGCCCC GAGGCTCTGAAGGTCCCCAGGGTGTGCG TGGTGAGCCTGGCCCCCCTGGCCCTGCT GGTGCTGCTGGCCCTGCTGGAAACCCTG GTGCTGATGGACAGCCTGGTGCTAAAGG TGCCAATGGTGCTCCTGGTATTGCTGGTG CTCCTGGCTTCCCTGGTGCCCGAGGCCC

[0763]

SEQ ID NO:	描述	序列
		CTCTGGACCCCAGGGCCCCGGCGGCCCT CCTGGTCCCAAGGGTAACAGCGGTGAAC CTGGTGCTCCTGGCAGCAAAGGAGACAC TGGTGCTAAGGGAGAGCCTGGCCCTGTT GGTGTTCAAGGACCCCCTGGCCCTGCTG GAGAGGAAGGAAAGCGAGGAGCTCGAG GTGAACCCGGACCCACTGGCCTGCCCGG ACCCCCTGGCGAGCGTGGTGGACCTGGT AGCCGTGGTTTCCCTGGCGCAGATGGTG TTGCTGGTCCCAAGGGTCCCGCTGGTGA ACGTGGTTCTCCTGGCCCTGCTGGCCCC AAAGGATCTCCTGGTGAAGCTGGTCGTC CCGGTGAAGCTGGTCTGCCTGGTGCCAA GGGTCTGACTGGAAGCCCTGGCAGCCCT GGTCCTGATGGCAAAACTGGCCCCCCTG GTCCCGCCGGTCAAGATGGTCGCCCCGG ACCCCCAGGCCCACCTGGTGCCCGTGGT CAGGCTGGTGTGATGGGATTCCCTGGAC CTAAAGGTGCTGCTGGAGAGCCCGGCAA GGCTGGAGAGCGAGGTGTTCCCGGACCC CCTGGCGCTGTCGGTCCTGCTGGCAAAG ATGGAGAGGCTGGAGCTCAGGGACCCCC TGGCCCTGCTGGTCCCGCTGGCGAGAGA GGTGAACAAGGCCCTGCTGGCTCCCCCG GATTCCAGGGTCTCCCTGGTCCTGCTGGT CCTCCAGGTGAAGCAGGCAAACCTGGTG AACAGGGTGTTCCTGGAGACCTTGGCGC CCCTGGCCCCTCTGGAGCAAGAGGCGAG AGAGGTTTCCCTGGCGAGCGTGGTGTGC AAGGTCCCCCTGGTCCTGCTGGTCCCCG AGGGGCCAACGGTGCTCCCGGCAACGAT GGTGCTAAGGGTGATGCTGGTGCCCCTG GAGCTCCCGGTAGCCAGGGCGCCCCTGG CCTTCAGGGAATGCCTGGTGAACGTGGT GCAGCTGGTCTTCCAGGGCCTAAGGGTG ACAGAGGTGATGCTGGTCCCAAAGGTGC TGATGGCTCTCCTGGCAAAGATGGCGTC CGTGGTCTGACTGGCCCCATTGGTCCTCC TGGCCCTGCTGGTGCCCCTGGTGACAAG GGTGAAAGTGGTCCCAGCGGCCCTGCTG

[0764]

SEQ ID NO:	描述	序列
		GTCCCACTGGAGCTCGTGGTGCCCCCGG AGACCGTGGTGAGCCTGGTCCCCCCGGC CCTGCTGGCTTTGCTGGCCCCCCTGGTGC TGACGGCCAACCTGGTGCTAAAGGCGAA CCTGGTGATGCTGGTGCTAAAGGCGATG CTGGTCCCCCTGGCCCTGCCGGACCCGC TGGACCCCCTGGCCCCATTGGTAATGTTG GTGCTCCTGGAGCCAAAGGTGCTCGCGG CAGCGCTGGTCCCCCTGGTGCTACTGGTT TCCCTGGTGCTGCTGGCCGAGTCGGTCC TCCTGGCCCCTCTGGAAATGCTGGACCC CCTGGCCCTCCTGGTCCTGCTGGCAAAG AAGGCGGCAAAGGTCCCCGTGGTGAGA CTGGCCCTGCTGGACGTCCTGGTGAAGT TGGTCCCCCTGGTCCCCCTGGCCCTGCTG GCGAGAAAGGATCCCCTGGTGCTGATGG TCCTGCTGGTGCTCCTGGTACTCCCGGGC CTCAAGGTATTGCTGGACAGCGTGGTGT GGTCGGCCTGCCTGGTCAGAGAGGAGAG AGAGGCTTCCCTGGTCTTCCTGGCCCCTC TGGTGAACCTGGCAAACAAGGTCCCTCT GGAGCAAGTGGTGAACGTGGTCCCCCTG GTCCCATGGGCCCCCCTGGATTGGCTGG ACCCCCTGGTGAATCTGGACGTGAGGGG GCTCCTGGTGCCGAAGGTTCCCCTGGAC GAGACGGTTCTCCTGGCGCCAAGGGTGA CCGTGGTGAGACCGGCCCCGCTGGACCC CCTGGTGCTCCTGGTGCTCCTGGTGCCCC TGGCCCCGTTGGCCCTGCTGGCAAGAGT GGTGATCGTGGTGAGACTGGTCCTGCTG GTCCCGCCGGTCCTGTCGGCCCTGTTGG CGCCCGTGGCCCCGCCGGACCCCAAGGC CCCCGTGGTGACAAGGGTGAGACAGGC GAACAGGGCGACAGAGGCATAAAGGGT CACCGTGGCTTCTCTGGCCTCCAGGGTC CCCCTGGCCCTCCTGGCTCTCCTGGTGAA CAAGGTCCCTCTGGAGCCTCTGGTCCTG CTGGTCCCCGAGGTCCCCCTGGCTCTGCT GGTGCTCCTGGCAAAGATGGACTCAACG GTCTCCCTGGCCCCATTGGGCCCCCTGGT

[0765]

SEQ ID NO:	描述	序列
		CCTCGCGGTCGCACTGGTGATGCTGGTC CTGTTGGTCCCCCCGGCCCTCCTGGACCT CCTGGTCCCCCTGGTCCTCCCAGCGCTG GTTTCGACTTCAGCTTCCTGCCCCAGCCA CCTCAAGAGAAGGCTCACGATGGTGGCC GCTACTACCGGGCTGATGATGCCAATGTG GTTCGTGACCGTGACCTCGAGGTGGACA CCACCCTCAAGAGCCTGAGCCAGCAGAT CGAGAACATCCGGAGCCCAGAGGGCAG CCGCAAGAACCCCGCCCGCACCTGCCGT GACCTCAAGATGTGCCACTCTGACTGGA AGAGTGGAGAGTACTGGATTGACCCCAA CCAAGGCTGCAACCTGGATGCCATCAAA GTCTTCTGCAACATGGAGACTGGTGAGA CCTGCGTGTACCCCACTCAGCCCAGTGT GGCCCAGAAGAACTGGTACATCAGCAAG AACCCCAAGGACAAGAGGCATGTCTGGT TCGGCGAGAGCATGACCGATGGATTCCA GTTCGAGTATGGCGGCCAGGGCTCCGAC CCTGCCGATGTGGCCATCCAGCTGACCTT CCTGCGCCTGATGTCCACCGAGGCCTCC CAGAACATCACCTACCACTGCAAGAACA GCGTGGCCTACATGGACCAGCAGACTGG CAACCTCAAGAAGGCCCTGCTCCTCCAG GGCTCCAACGAGATCGAGATCCGCGCCG AGGGCAACAGCCGCTTCACCTACAGCGT CACTGTCGATGGCTGCACGAGTCACACC GGAGCCTGGGGCAAGACAGTGATTGAAT ACAAAACCACCAAGACCTCCCGCCTGCC CATCATCGATGTGGCCCCCTTGGACGTTG GTGCCCCAGACCAGGAATTCGGCTTCGA CGTTGGCCCTGTCTGCTTCCTGTAAACTC CCTCCATCCCAACCTGGCTCCCTCCCACC CAACCAACTTTCCCCCCAACCCGGAAAC AGACAAGCAACCCAAACTGAACCCCCTC AAAAGCCAAAAAATGGGAGACAATTTCA CATGGACTTTGGAAAATATTTTTTCCTTT GCATTCATCTCTCAAACTTAGTTTTTATCT TTGACCAACCGAACATGACCAAAAACCA AAAGTGCATTCAACCTTACCAAAAAAAA

[0766]

SEQ ID NO:	描述	序列
		AAAAAAAAAAAGAATAAATAAATAACTT TTTAAAAAAGGAAGCTTGGTCCACTTGC TTGAAGACCCATGCGGGGGTAAGTCCCT TTCTGCCCGTTGGGCTTATGAAACCCCAA TGCTGCCCTTTCTGCTCCTTTCTCCACAC CCCCCTTGGGGCCTCCCCTCCACTCCTTC CCAAATCTGTCTCCCCAGAAGACACAGG AAACAATGTATTGTCTGCCCAGCAATCAA AGGCAATGCTCAAACACCCAAGTGGCCC CCACCCTCAGCCCGCTCCTGCCCGCCCA GCACCCCCAGGCCCTGGGGGACCTGGGG TTCTCAGACTGCCAAAGAAGCCTTGCCA TCTGGCGCTCCCATGGCTCTTGCAACATC TCCCCTTCGTTTTTGAGGGGGTCATGCCG GGGGAGCCACCAGCCCCTCACTGGGTTC GGAGGAGAGTCAGGAAGGGCCACGACA AAGCAGAAACATCGGATTTGGGGAACGC GTGTCAATCCCTTGTGCCGCAGGGCTGG GCGGGAGAGACTGTTCTGTTCCTTGTGTA ACTGTGTTGCTGAAAGACTACCTCGTTCT TGTCTTGATGTGTCACCGGGGCAACTGC CTGGGGGCGGGGATGGGGGCAGGGTGG AAGCGGCTCCCCATTTTATACCAAAGGTG CTACATCTATGTGATGGGTGGGGTGGGGA GGGAATCACTGGTGCTATAGAAATTGAGA TGCCCCCCCAGGCCAGCAAATGTTCCTTT TTGTTCAAAGTCTATTTTTATTCCTTGATA TTTTTCTTTTTTTTTTTTTTTTTTTGTGGAT GGGGACTTGTGAATTTTTCTAAAGGTGCT ATTTAACATGGGAGGAGAGCGTGTGCGG CTCCAGCCCAGCCCGCTGCTCACTTTCCA CCCTCTCTCCACCTGCCTCTGGCTTCTCA GGCCTCTGCTCTCCGACCTCTCTCCTCTG AAACCCTCCTCCACAGCTGCAGCCCATC CTCCCGGCTCCCTCCTAGTCTGTCCTGCG TCCTCTGTCCCCGGGTTTCAGAGACAAC TTCCCAAAGCACAAAGCAGTTTTTCCCC CTAGGGGTGGGAGGAAGCAAAAGACTCT GTACCTATTTTGTATGTGTATAATAATTTG AGATGTTTTTAATTATTTTGATTGCTGGAA

[0767]

SEQ ID NO:	描述	序列
		TAAAGCATGTGGAAATGACCCAAACATAA
77	激活素 A mRNA	ATGCCCTTGCTTTGGCTGAGAGGATTTCTGTTGGCAAGTTGCTGGATTATAGTGAGGAGTTCCCCCACCCCAGGATCCGAGGGGCACAGCGCGGCCCCCGACTGTCCGTCCTGTGCGCTGGCCGCCCTCCCAAAGGATGTACCCAACTCTCAGCCAGAGATGGTGGAGGCCGTCAAGAAGCACATTTTAAACATGCTGCACTTGAAGAAGAGACCCGATGTCACCCAGCCGGTACCCAAGGCGGCGCTTCTGAACGCGATCAGAAAGCTTCATGTGGGCAAAGTCGGGGAGAACGGGTATGTGGAGATAGAGGATGACATTGGAAGGAGGGCAGAAATGAATGAACTTATGGAGCAGACCTCGGAGATCATCACGTTTGCCGAGTCAGGTTGGTGCTGGCATTGGCAGGGGGTGGGGAGGGGTGGGGGGTGGGAGGGTAAAATATATTTCTTTGACAGTCCCAGGAGGAACTTCTTTTCCCTTCAGCTGGAAACTGCCTGGGAAGGTTATTAGTTATTAGGTGATGGTAGCGGACTAGCCGACGGAGGGCAGGCAGGGGAGGGGGAGAGGACTTTACAGAAAAGGAATTCTCGGTCGAGCTCTGCCTGGAGATGACTGGCTTACACTTACTAAACCCAGCGGGTCACACAGAGAGGAAGCTCGGGGCCAATGTTGAGCTGGAAGGCAGACTGTGAGGGGCTGCCTTGCCCTGCCTGTGAAACAGATCTGAGCAGCCGGAGGAAAGCCGCGGCATTTTCGGGTGCTAGGGGAGCAGAGGAGGCTTCCGGACCCCATCCAAGTTTTTATTGAGGGTAGAGGGGTGAATGTACCAGGATTGGAGTGGAATGGCACAGATGAAGTCACTCTCTTAAAACAAACCTTCCCCTTTAAAAGTCCAATCTGGGGCCACATTGGAGAAGCAGGGCATATTTATGAGTGACAGTCATTTTTACCTTTAGAAAATGTCTATAAGTGCACAGGCACCACATTCAAGACAGGGAAGAGCTACTTTGGGGGACAGTTGTCATTGAACCAGCAGTTACTTTTG

[0768]

SEQ ID NO:	描述	序列
		GGACACTGACTTTTGCTCTCTGAAAGAA AAAAAAATAAATAAAACAACCAGTTTTG TTCTTTCTAAAGTTACTAAGAGCTCTCTG CCAAGGAACGAACCTTGACAAAGTACTC TCAGATACTACGCTGAAGTCACTCAATCT TAAGAGGAAGAAG
78	Snai1 引物 1	TCGGAAGCCTAACTACAGCGA
79	Snai1 引物 2	AGATGAGCATTGGCAGCGAG
80	Dkk1 引物 1	CCTTGAACTCGGTTCTCAATTCC
81	Dkk1 引物 2	CAATGGTCTGGTACTTATTCCCG
82	Col1a1 引物 1	GAGGGCCAAGACGAAGACATC
83	Col1a1 引物 2	CAGATCACGTCATCGCACAAC
84	激活素引物 1	TCATCACGTTTGCCGAGTCA
85	激活素引物 2	GCTAGTCCGCTACCATCACC
86	人类 Axin2 mRNA	ACTGGCTCCGCGAGCCTGGCCCGGGGGA GTCGGCTGGAGCCGGCTGCGCTTTGATA AGGTCCTGGCAACTCAGTAACAGCCCGA GAGCCGGGAAATAAAAATAACCCCTCAG AGCGATGGATTTCGGGGCCGCCCGGCGG CCGAGGCGCCCGCCGAAGGCCCTGCTGT AAAAGAGAGGAGGTTCAGATGAGCCCCT GCTGACTTGAGAGAGACAGAGAGACCA CGCCGATTGCTGAGAGGAACTGGAAGAA GAAAAATTCCCAGACTCAGTGGGAAGAG CTCCCTCACCATGAGTAGCGCTATGTTGG TGACTTGCCTCCCGGACCCCAGCAGCAG CTTCCGTGAGGATGCCCCGCGGCCCCCA GTGCCAGGGGAAGAAGGGGAGACCCCA CCGTGTCAGCCAGGGGTGGGCAAGGGCC AGGTCACCAAACCCATGCCTGTCTCTTCC AACACCAGGCGGAACGAAGATGGGTTG GGGGAGCCGGAGGGGCGGGCATCTCCG GATTCCCCTCTGACCCGGTGGACCAAGT CCTTACACTCCTTATTGGGCGATCAAGAC GGTGCTTACCTGTTCCGAACTTTCCTGGA GAGGGAGAAATGCGTGGATACCTTAGAC TTCTGGTTTGCCTGCAATGGATTCAGGCA

[0769]

SEQ ID NO:	描述	序列
		GATGAACCTGAAGGATACCAAAACTTTA CGAGTAGCCAAAGCGATCTACAAAAGGT ACATTGAGAACAACAGCATTGTCTCCAA GCAGCTGAAGCCTGCCACCAAGACCTAC ATAAGAGATGGCATCAAGAAGCAGCAGA TTGATTCCATCATGTTTGACCAGGCGCAG ACCGAGATCCAGTCGGTGATGGAGGAAA ATGCCTACCAGATGTTTTTGACTTCTGATA TATACCTCGAATATGTGAGGAGTGGGGGA GAAAACACAGCTTACATGAGTAATGGGG GACTCGGGAGCCTAAAGGTCGTGTGTGG CTATCTCCCCACCTTGAATGAAGAAGAG GAGTGGACTTGTGCCGACTTCAAGTGCA AACTTTCGCCAACCGTGGTTGGCTTGTCC AGCAAAACTCTGAGGGCCACGGCGAGT GTGAGGTCCACGGAAACTGTTGACAGTG GATACAGGTCCTTCAAGAGGAGCGATCC TGTTAATCCTTATCACATAGGTTCTGGCTA TGTCTTTGCACCAGCCACCAGCGCCAAC GACAGTGAGATATCCAGTGATGCGCTGA CGGATGATTCCATGTCCATGACGGACAGC AGTGTAGATGGAATTCCTCCTTATCGTGT GGGCAGTAAGAAACAGCTCCAGAGAGA AATGCATCGCAGTGTGAAGGCCAATGGC CAAGTGTCTCTACCTCATTTCCCGAGAAC CCACCGCCTGCCCAAGGAGATGACCCCC GTGGAACCCGCCACCTTTGCAGCTGAGC TGATCTCGAGGCTGGAAAAGCTGAAGCT GGAGTTGGAGAGCCGCCACAGCCTGGA GGAGCGCCTGCAGCAGATCCGAGAGGAT GAAGAGAGAGAGGGCTCCGAGCTCACA CTCAATTCGCGGGAGGGGGCGCCCACGC AGCACCCCCTCTCCCTACTGCCCTCCGGC AGCTACGAGGAAGACCCGCAGACGATAC TGGACGATCACCTGTCCAGGGTCCTCAA GACCCCTGGCTGCCAGTCTCCAGGCGTA GGCCGCTATAGCCCCCGCTCCCGCTCCCC GGACCACCACCACCACCACCATTCGCAG TACCACTCCCTGCTCCCGCCCGGTGGCA AGCTGCCTCCCGCGGCCGCCTCGCCGGG

[0770]

SEQ ID NO:	描述	序列
		CGCCTGCCCCCTCCTCGGGGGCAAAGGC TTTGTGACCAAGCAGACGACGAAGCATG TCCACCACCACTACATCCACCACCATGCC GTCCCCAAGACCAAGGAGGAGATCGAG GCGGAGGCCACGCAGCGGGTGCACTGCT TCTGCCCTGGGGGCAGCGAGTATTACTGC TACTCGAAATGCAAAAGCCACTCCAAGG CTCCGGAAACCATGCCCAGCGAGCAGTT TGGCGGCAGCAGAGGCAGTACCTTGCCC AAACGCAATGGGAAAGGCACGGAGCCG GGCCTGGCCCTGCCCGCCAGGGAAGGAG GGGCCCCCGGCGGAGCTGGGGCCCTGCA GCTTCCCCGGGAGGAAGGAGACAGGTC GCAGGATGTCTGGCAGTGGATGCTGGAG AGTGAGCGGCAGAGCAAGCCCAAGCCC CATAGTGCCCAAAGCACAAAAAAGGCCT ACCCCTTGGAGTCTGCCCGCTCGTCTCCA GGCGAACGAGCCAGCCGGCACCATCTGT GGGGGGGCAACAGCGGGCACCCCCGCA CCACCCCCCGTGCCCACCTGTTCACCCA GGACCCTGCGATGCCTCCCCTGACCCCA CCCAACACGCTGGCTCAGCTGGAGGAGG CCTGTCGCAGGCTAGCTGAGGTGTCGAA GCCCCCAAAGCAGCGGTGCTGTGTGGCC AGTCAGCAGAGGGACAGGAATCATTCGG CCACTGTTCAGACGGGAGCCACACCCTT CTCCAATCCAAGCCTGGCTCCAGAAGAT CACAAAGAGCCAAAGAAACTGGCAGGT GTCCACGCGCTCCAGGCCAGTGAGTTGG TTGTCACTTACTTTTTCTGTGGGGAAGAA ATTCCATACCGGAGGATGCTGAAGGCTCA GAGCTTGACCCTGGGCCACTTTAAAGAG CAGCTCAGCAAAAAGGGAAATTATAGGT ATTACTTCAAAAAAGCAAGCGATGAGTT TGCCTGTGGAGCGGTGTTTGAGGAGATC TGGGAGGATGAGACGGTGCTCCCGATGT ATGAAGGCCGGATTCTGGGCAAAGTGGA GCGGATCGATTGAGCCCTGGGGTCTGGC TTTGGTGAACTGTTGGAGCCCGAAGCTC TTGTGAACTGTCTTGGCTGTGAGCAACT

[0771]

SEQ ID NO:	描述	序列
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[0772]

SEQ ID NO:	描述	序列
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87	人类 Axin2 氨基酸序列	MSSAMLVTCLPDPSSSFREDAPRPPVPGEE GETPPCQPGVGKGQVTKPMPVSSNTRRNE DGLGEPEGRASPDSPLTRWTKSLHSLLGDQ DGAYLFRTFLEREKCVDTLDFWFACNGFR QMNLKDTKTLRVAKAIYKRYIENNSIVSKQ LKPATKTYIRDGIKKQQIDSIMFDQAQTEIQ SVMEENAYQMFLTSDIYLEYVRSGGENTA YMSNGGLGSLKVVCGYLPTLNEEEEWTC ADFKCKLSPTVVGLSSKTLRATASVRSTET VDSGYRSFKRSDPVNPYHIGSGYVFAPATS ANDSEISSDALTDDSMSMTDSSVDGIPPYR VGSKKQLQREMHRSVKANGQVSLPHFPRT HRLPKEMTPVEPATFAAELISRLEKLKLELE SRHSLEERLQQIREDEEREGSELTLNSREG APTQHPLSLLPSGSYEEDPQTILDDHLSRVL KTPGCQSPGVGRYSPRSRSPDHHHHHHSQ YHSLLPPGGKLPPAAASPGACPLLGGKGFV TKQTTKHVHHHYIHHHAVPKTKEEIEAEA TQRVHCFCPGGSEYYCYSKCKSHSKAPET MPSEQFGGSRGSTLPKRNGKGTEPGLALP AREGGAPGGAGALQLPREEGDRSQDVWQ WMLESERQSKPKPHSAQSTKKAYPLESAR SSPGERASRHHLWGGNSGHPRTTPRAHLF TQDPAMPPLTPPNTLAQLEEACRRLAEVSK PPKQRCCVASQQRDRNHSATVQTGATPFS NPSLAPEDHKEPKKLAGVHALQASELVVT YFFCGEEIPYRRMLKAQSLTLGHFKEQLSK KGNYRYYFKKASDEFACGAVFEEIWEDET VLPMYEGRILGKVERID

[0773] 11.等同

[0774] 虽然参考具体实施方式详细描述了本发明，要理解的是功能上等价的变体处于本发明的范围之内。实际上，根据以上的说明和附图，除了在此显示和描述的之外，本发明的各种修饰对于本领域技术人员而言是显而易见的。这种修改也将落在附随的权利要求的范

围之中。本领域的技术人员将认识到或能够利用不超出常规的实验来确定本文描述的本发明的具体实施方式的许多等同形式。这样的等同形式预期由以下权利要求涵盖。

[0775] 在本说明书中提及的所有出版物、专利和专利申请通过引用合并到本说明书中，达到如同每个单独的出版物、专利或专利申请被具体和分别地指明通过引用完全合并在此的相同程度。

序列表

<110> 细胞基因公司

华盛顿大学

<120> 利用ACTRII配体陷阱治疗心血管疾病

<130> 12827-934-228

<150> 62/062,021

<151> 2014-10-09

<150> 62/078,321

<151> 2014-11-11

<150> 62/103,515

<151> 2015-01-14

<150> 62/167,052

<151> 2015-05-27

<150> 62/170,015

<151> 2015-06-02

<160> 87

<170> PatentIn version 3.5

<210> 1

<211> 513

<212> PRT

<213> 智人

<220>

<223> 人ActRIIA前体多肽

<400> 1

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20 25 30
Phe Asn Ala Asn Trp Glu Lys Asp Arg Thr Asn Gln Thr Gly Val Glu
35 40 45
Pro Cys Tyr Gly Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp
50 55 60
Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu
65 70 75 80
Asp Asp Ile Asn Cys Tyr Asp Arg Thr Asp Cys Val Glu Lys Lys Asp
85 90 95
Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu
100 105 110

Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn
115 120 125
Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn Ile Leu Leu Tyr Ser Leu
130 135 140
Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala Phe Trp Val
145 150 155 160
Tyr Arg His His Lys Met Ala Tyr Pro Pro Val Leu Val Pro Thr Gln
165 170 175
Asp Pro Gly Pro Pro Pro Pro Ser Pro Leu Leu Gly Leu Lys Pro Leu
180 185 190
Gln Leu Leu Glu Val Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys
195 200 205
Ala Gln Leu Leu Asn Glu Tyr Val Ala Val Lys Ile Phe Pro Ile Gln
210 215 220
Asp Lys Gln Ser Trp Gln Asn Glu Tyr Glu Val Tyr Ser Leu Pro Gly
225 230 235 240
Met Lys His Glu Asn Ile Leu Gln Phe Ile Gly Ala Glu Lys Arg Gly
245 250 255
Thr Ser Val Asp Val Asp Leu Trp Leu Ile Thr Ala Phe His Glu Lys
260 265 270
Gly Ser Leu Ser Asp Phe Leu Lys Ala Asn Val Val Ser Trp Asn Glu
275 280 285
Leu Cys His Ile Ala Glu Thr Met Ala Arg Gly Leu Ala Tyr Leu His
290 295 300
Glu Asp Ile Pro Gly Leu Lys Asp Gly His Lys Pro Ala Ile Ser His
305 310 315 320
Arg Asp Ile Lys Ser Lys Asn Val Leu Leu Lys Asn Asn Leu Thr Ala
325 330 335
Cys Ile Ala Asp Phe Gly Leu Ala Leu Lys Phe Glu Ala Gly Lys Ser
340 345 350
Ala Gly Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro
355 360 365
Glu Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe Leu Arg
370 375 380
Ile Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Ala Ser Arg
385 390 395 400
Cys Thr Ala Ala Asp Gly Pro Val Asp Glu Tyr Met Leu Pro Phe Glu
405 410 415
Glu Glu Ile Gly Gln His Pro Ser Leu Glu Asp Met Gln Glu Val Val

420 425 430

Val His Lys Lys Lys Arg Pro Val Leu Arg Asp Tyr Trp Gln Lys His
435 440 445

Ala Gly Met Ala Met Leu Cys Glu Thr Ile Glu Glu Cys Trp Asp His
450 455 460

Asp Ala Glu Ala Arg Leu Ser Ala Gly Cys Val Gly Glu Arg Ile Thr
465 470 475 480

Gln Met Gln Arg Leu Thr Asn Ile Ile Thr Thr Glu Asp Ile Val Thr
485 490 495

Val Val Thr Met Val Thr Asn Val Asp Phe Pro Pro Lys Glu Ser Ser
500 505 510

Leu

<210> 2

<211> 115

<212> PRT

<213> 智人

<220>

<223> 人ActRIIA可溶的(细胞外的)，加工的多肽序列

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Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys Leu Phe Phe Asn Ala Asn
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Trp Glu Lys Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly
20 25 30

Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser
35 40 45

Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile Asn
50 55 60

Cys Tyr Asp Arg Thr Asp Cys Val Glu Lys Lys Asp Ser Pro Glu Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu Lys Phe Ser Tyr
85 90 95

Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn Pro Val Thr Pro
100 105 110

Lys Pro Pro
115

<210> 3

<211> 100

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIA可溶的(细胞外的)，C-末端15个氨基酸删除的加工的多肽序列

<400> 3

Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys Leu Phe Phe Asn Ala Asn
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Trp Glu Lys Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly
20 25 30
Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser
35 40 45
Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile Asn
50 55 60
Cys Tyr Asp Arg Thr Asp Cys Val Glu Lys Lys Asp Ser Pro Glu Val
65 70 75 80
Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu Lys Phe Ser Tyr
85 90 95
Phe Pro Glu Met
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<210> 4

<211> 1542

<212> DNA

<213> 智人

<220>

<223> 编码人ActRIIA前体蛋白的核酸序列

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atacttggta gatcagaaac tcaggagtgt cttttcttta atgctaattg ggaaaaagac 120
agaaccaatc aaactggtgt tgaaccgtgt tatggtgaca aagataaacg gcggcattgt 180
tttgctacct ggaagaatat ttctggttcc attgaaatag tgaaacaagg ttgttggctg 240
gatgatatca actgctatga caggactgat tgtgtagaaa aaaaagacag ccctgaagta 300
tatttttgtt gctgtgaggg caatatgtgt aatgaaaagt tttcttattt tccagagatg 360
gaagtcacac agcccacttc aaatccagtt acacctaagc caccctatta caacatcctg 420
ctctattcct tggtgccact tatgttaatt gcggggattg tcatttgtgc attttgggtg 480
tacaggcatc acaagatggc ctaccctcct gtacttgttc caactcaaga cccaggacca 540
cccccacctt ctccattact agggttgaaa ccactgcagt tattagaagt gaaagcaagg 600
ggaagatttg gttgtgtctg gaaagcccag ttgcttaacg aatatgtggc tgtcaaaata 660
tttccaatac aggacaaaca gtcatggcaa aatgaatacg aagtctacag tttgcctgga 720
atgaagcatg agaacatatt acagttcatt ggtgcagaaa aacgaggcac cagtgttgat 780
gtggatcttt ggctgatcac agcatttcat gaaaagggtt cactatcaga ctttcttaag 840

gctaatgtgg tctcttggaa tgaactgtgt catattgcag aaaccatggc tagaggattg 900
gcatatttac atgaggatat acctggccta aaagatggcc acaaacctgc catatctcac 960
agggacatca aaagtaaaaa tgtgctgttg aaaaacaacc tgacagcttg cattgctgac 1020
tttgggttgg ccttaaaatt tgaggctggc aagtctgcag gcgataccca tggacaggtt 1080
ggtacccgga ggtacatggc tccagaggta ttagagggtg ctataaactt cgaaagggat 1140
gcatttttga ggatagatat gtatgccatg ggattagtcc tatgggaact ggcttctcgc 1200
tgtactgctg cagatggacc tgtagatgaa tacatgttgc catttgagga ggaaattggc 1260
cagcatccat ctcttgaaga catgcaggaa gttgttgtgc ataaaaaaaa gaggcctgtt 1320
ttaagagatt attggcagaa acatgctgga atggcaatgc tctgtgaaac cattgaagaa 1380
tgttgggatc acgacgcaga agccaggtta tcagctggat gtgtaggtga aagaattacc 1440
cagatgcaga gactaacaaa tattattacc acagaggaca ttgtaacagt ggtcacaatg 1500
gtgacaaatg ttgactttcc tcccaaagaa tctagtctat ga 1542

<210> 5
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<212> DNA
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<220>
<223> 编码人ActRIIA可溶的(细胞外的)多肽的核酸序列
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agaaccaatc aaactggtgt tgaaccgtgt tatggtgaca aagataaacg gcggcattgt 120
tttgctacct ggaagaatat ttctggttcc attgaaatag tgaaacaagg ttgttggctg 180
gatgatatca actgctatga caggactgat tgtgtagaaa aaaaagacag ccctgaagta 240
tatttttgtt gctgtgaggg caatatgtgt aatgaaaagt tttcttattt tccagagatg 300
gaagtcacac agcccacttc aaatccagtt acacctaagc caccc 345

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<211> 228
<212> PRT
<213> 人工序列
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<223> 包含与Fc结构域融合的ActRIIA的可溶细胞外结构域的融合蛋白
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<223> Xaa = Asp或Ala
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<400> 6

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1 5 10 15
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
20 25 30
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Xaa Val Ser His Glu
35 40 45
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
50 55 60
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
65 70 75 80
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
85 90 95
Glu Tyr Lys Cys Lys Xaa Val Ser Asn Lys Ala Leu Pro Val Pro Ile
100 105 110
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
130 135 140
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145 150 155 160
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175
Val Leu Asp Ser Asp Gly Pro Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
195 200 205
His Glu Ala Leu His Asn Xaa His Tyr Thr Gln Lys Ser Leu Ser Leu
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Ser Pro Gly Lys
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<210> 7
<211> 344
<212> PRT
<213> 人工序列

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<223> 与人Fc结构域融合的人ActRIIA的细胞外结构域

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Trp Glu Lys Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly
20 25 30
Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser
35 40 45
Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile Asn
50 55 60
Cys Tyr Asp Arg Thr Asp Cys Val Glu Lys Lys Asp Ser Pro Glu Val
65 70 75 80
Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu Lys Phe Ser Tyr
85 90 95
Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn Pro Val Thr Pro
100 105 110
Lys Pro Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala
115 120 125
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
130 135 140
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
145 150 155 160
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
165 170 175
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
180 185 190
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
195 200 205
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
210 215 220
Leu Pro Val Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
225 230 235 240
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
245 250 255
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
260 265 270
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
275 280 285

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
290 295 300
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
305 310 315 320
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
325 330 335
Ser Leu Ser Leu Ser Pro Gly Lys
340

<210> 8
<211> 21
<212> PRT
<213> 人工序列
<220>
<223> 蜜蜂蜂毒(HBML)的前导序列
<400> 8
Met Lys Phe Leu Val Asn Val Ala Leu Val Phe Met Val Val Tyr Ile
1 5 10 15
Ser Tyr Ile Tyr Ala
20

<210> 9
<211> 22
<212> PRT
<213> 人工序列
<220>
<223> 组织纤溶酶原激活物(TPA)的前导序列
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Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15
Ala Val Phe Val Ser Pro
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<210> 10
<211> 20
<212> PRT
<213> 未知
<220>
<223> 天然ActRIIA前导区
<400> 10
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1 5 10 15

Ser Ser Gly Ala
20

<210> 11
<211> 9
<212> PRT
<213> 人工序列
<220>
<223> ActRIIA-hFc和mActRIIA-Fc N-末端序列
<400> 11
Ile Leu Gly Arg Ser Glu Thr Gln Glu
1 5

<210> 12
<211> 329
<212> PRT
<213> 人工序列
<220>
<223> 具有ActRIIA的细胞外结构域的C-末端15个氨基酸删除的ActRIIA-Fc蛋白
<400> 12
Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys Leu Phe Phe Asn Ala Asn
1 5 10 15
Trp Glu Lys Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly
20 25 30
Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser
35 40 45
Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile Asn
50 55 60
Cys Tyr Asp Arg Thr Asp Cys Val Glu Lys Lys Asp Ser Pro Glu Val
65 70 75 80
Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu Lys Phe Ser Tyr
85 90 95
Phe Pro Glu Met Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro
100 105 110
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
115 120 125
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
130 135 140
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
145 150 155 160
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu

165 170 175

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
180 185 190

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
195 200 205

Ala Leu Pro Val Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
210 215 220

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
225 230 235 240

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
245 250 255

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
260 265 270

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
275 280 285

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
290 295 300

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
305 310 315 320

Lys Ser Leu Ser Leu Ser Pro Gly Lys
325

<210> 13
<211> 369
<212> PRT
<213> 人工序列
<220>
<223> 带有TPA前导序列的未加工的ActRIIA-hFc
<400> 13

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Gly Ala Ala Ile Leu Gly Arg Ser Glu Thr
20 25 30

Gln Glu Cys Leu Phe Phe Asn Ala Asn Trp Glu Lys Asp Arg Thr Asn
35 40 45

Gln Thr Gly Val Glu Pro Cys Tyr Gly Asp Lys Asp Lys Arg Arg His
50 55 60

Cys Phe Ala Thr Trp Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys
65 70 75 80

Gln Gly Cys Trp Leu Asp Asp Ile Asn Cys Tyr Asp Arg Thr Asp Cys

85 90 95

Val Glu Lys Lys Asp Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly
100 105 110

Asn Met Cys Asn Glu Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr
115 120 125

Gln Pro Thr Ser Asn Pro Val Thr Pro Lys Pro Pro Thr Gly Gly Gly
130 135 140

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
145 150 155 160

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
165 170 175

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
180 185 190

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
195 200 205

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
210 215 220

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
225 230 235 240

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Val Pro Ile Glu Lys
245 250 255

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
260 265 270

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
275 280 285

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
290 295 300

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
305 310 315 320

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
325 330 335

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
340 345 350

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
355 360 365

Lys

<210> 14

<211> 1113

<212> DNA

<213> 人工序列

<220>

<223> 编码带有TPA前导序列的未加工的ActRIIA-hFc的核酸序列

<400> 14

atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt 60
tcgcccggcg ccgctatact tggtagatca gaaactcagg agtgtctttt tttaatgcta 120
attgggaaaa agacagaacc aatcaaactg gtgttgaacc gtgttatggt gacaaagata 180
aacggcggca ttgttttgct acctggaaga atatttctgg ttccattgaa tagtgaaaca 240
aggttgttgg ctggatgata tcaactgcta tgacaggact gattgtgtag aaaaaaaaga 300
cagccctgaa gtatatttct gttgctgtga gggcaatatg tgtaatgaaa agttttctta 360
ttttccggag atggaagtca cacagcccac ttcaaatcca gttacaccta agccacccac 420
cggtggtgga actcacacat gcccaccgtg cccagcacct gaactcctgg ggggaccgtc 480
agtcttcctc ttcccccccaa aacccaagga caccctcatg atctcccgga cccctgaggt 540
cacatgcgtg gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt 600
ggacggcgtg gaggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac 660
gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta 720
caagtgcaag gtctccaaca aagccctccc agtccccatc gagaaaacca tctccaaagc 780
caaagggcag ccccgagaac cacaggtgta caccctgccc ccatcccggg aggagatgac 840
caagaaccag gtcagcctga cctgcctggt caaaggcttc tatcccagcg acatcgccgt 900
ggagtgggag agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga 960
ctccgacggc tccttcttcc tctatagcaa gctcaccgtg gacaagagca ggtggcagca 1020
ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa 1080
gagcctctcc ctgtctccgg taaatgagaa ttc 1113

<210> 15

<211> 106

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端4个氨基酸删除(SEQ ID NO:28的氨基酸25-130)、具有L79D突变的加工的多肽序列

<400> 15

Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15
Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45
Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro
100 105

<210> 16
<211> 512
<212> PRT
<213> 智人
<220>
<223> 人ActRIIB前体蛋白序列(A64)
<400> 16

Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Trp
1 5 10 15
Pro Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
20 25 30
Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
35 40 45
Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala
50 55 60
Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65 70 75 80
Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
85 90 95
Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
100 105 110
Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
115 120 125
Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
130 135 140
Pro Ile Gly Gly Leu Ser Leu Ile Val Leu Leu Ala Phe Trp Met Tyr
145 150 155 160
Arg His Arg Lys Pro Pro Tyr Gly His Val Asp Ile His Glu Asp Pro
165 170 175
Gly Pro Pro Pro Pro Ser Pro Leu Val Gly Leu Lys Pro Leu Gln Leu
180 185 190
Leu Glu Ile Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln
195 200 205

Leu Met Asn Asp Phe Val Ala Val Lys Ile Phe Pro Leu Gln Asp Lys
210 215 220
Gln Ser Trp Gln Ser Glu Arg Glu Ile Phe Ser Thr Pro Gly Met Lys
225 230 235 240
His Glu Asn Leu Leu Gln Phe Ile Ala Ala Glu Lys Arg Gly Ser Asn
245 250 255
Leu Glu Val Glu Leu Trp Leu Ile Thr Ala Phe His Asp Lys Gly Ser
260 265 270
Leu Thr Asp Tyr Leu Lys Gly Asn Ile Ile Thr Trp Asn Glu Leu Cys
275 280 285
His Val Ala Glu Thr Met Ser Arg Gly Leu Ser Tyr Leu His Glu Asp
290 295 300
Val Pro Trp Cys Arg Gly Glu Gly His Lys Pro Ser Ile Ala His Arg
305 310 315 320
Asp Phe Lys Ser Lys Asn Val Leu Leu Lys Ser Asp Leu Thr Ala Val
325 330 335
Leu Ala Asp Phe Gly Leu Ala Val Arg Phe Glu Pro Gly Lys Pro Pro
340 345 350
Gly Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu
355 360 365
Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe Leu Arg Ile
370 375 380
Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Val Ser Arg Cys
385 390 395 400
Lys Ala Ala Asp Gly Pro Val Asp Glu Tyr Met Leu Pro Phe Glu Glu
405 410 415
Glu Ile Gly Gln His Pro Ser Leu Glu Glu Leu Gln Glu Val Val Val
420 425 430
His Lys Lys Met Arg Pro Thr Ile Lys Asp His Trp Leu Lys His Pro
435 440 445
Gly Leu Ala Gln Leu Cys Val Thr Ile Glu Glu Cys Trp Asp His Asp
450 455 460
Ala Glu Ala Arg Leu Ser Ala Gly Cys Val Glu Glu Arg Val Ser Leu
465 470 475 480
Ile Arg Arg Ser Val Asn Gly Thr Thr Ser Asp Cys Leu Val Ser Leu
485 490 495
Val Thr Ser Val Thr Asn Val Asp Leu Pro Pro Lys Glu Ser Ser Ile
500 505 510

<210> 17

<211> 116

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),加工的多肽序列(SEQ ID NO:16的氨基酸19-134)

<400> 17

Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala
1 5 10 15
Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu
20 25 30
Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser
35 40 45
Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe
50 55 60
Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln
65 70 75 80
Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr
85 90 95
His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro
100 105 110
Thr Ala Pro Thr
115

<210> 18

<211> 101

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),C-末端15个氨基酸删除的加工的多肽序列(SEQ ID NO:16的氨基酸19-119)

<400> 18

Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala
1 5 10 15
Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu
20 25 30
Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser
35 40 45
Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe
50 55 60

Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln
65 70 75 80
Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr
85 90 95
His Leu Pro Glu Ala
100

<210> 19
<211> 1539
<212> DNA
<213> 智人
<220>
<223> 编码人ActRIIB (A64)前体蛋白的核酸序列
<400> 19

atgacggcgc cctgggtggc cctcgccctc ctctggggat cgctgtggcc cggctctggg 60
cgtggggagg ctgagacacg ggagtgcatc tactacaacg ccaactggga gctggagcgc 120
accaaccaga gcggcctgga gcgctgcgaa ggcgagcagg acaagcggct gcactgctac 180
gcctcctggg ccaacagctc tggcaccatc gagctcgtga agaagggctg ctggctagat 240
gacttcaact gctacgatag gcaggagtgt gtggccactg aggagaaccc ccaggtgtac 300
ttctgctgct gtgaaggcaa cttctgcaac gagcgcttca ctcatttgcc agaggctggg 360
ggcccggaag tcacgtacga gccacccccg acagccccca ccctgctcac ggtgctggcc 420
tactcactgc tgcccatcgg gggcctttcc ctcatcgtcc tgctggcctt ttggatgtac 480
cggcatcgca agccccccta cggtcatgtg gacatccatg aggaccctgg gcctccacca 540
ccatcccctc tggtgggcct gaagccactg cagctgctgg agatcaaggc tcgggggcgc 600
tttggctgtg tctggaaggc ccagctcatg aatgactttg tagctgtcaa gatcttccca 660
ctccaggaca agcagtcgtg gcagagtgaa cgggagatct tcagcacacc tggcatgaag 720
cacgagaacc tgctacagtt cattgctgcc gagaagcgag gctccaacct cgaagtagag 780
ctgtggctca tcacggcctt ccatgacaag ggctccctca cggattacct caaggggaac 840
atcatcacat ggaacgaact gtgtcatgta gcagagacga tgtcacgagg cctctcatac 900
ctgcatgagg atgtgccctg gtgccgtggc gagggccaca agccgtctat tgcccacagg 960
gactttaaaa gtaagaatgt attgctgaag agcgacctca cagccgtgct ggctgacttt 1020
ggcttggctg ttcgatttga gccagggaaa cctccagggg acacccacgg acaggtaggc 1080
acgagacggt acatggctcc tgaggtgctc gagggagcca tcaacttcca gagagatgcc 1140
ttcctgcgca ttgacatgta tgccatgggg ttggtgctgt gggagcttgt gtctcgctgc 1200
aaggctgcag acggacccgt ggatgagtac atgctgccct ttgaggaaga gattggccag 1260
caccccttcgt tggaggagct gcaggaggtg gtggtgcaca agaagatgag gcccaccatt 1320
aaagatcact ggttgaaaca cccgggcctg gcccagcttt gtgtgaccat cgaggagtgc 1380
tgggaccatg atgcagaggc tcgcttgtcc gcgggctgtg tggaggagcg ggtgtccctg 1440
attcggaggt cggtcaacgg cactacctcg gactgtctcg tttccctggt gacctctgtc 1500
accaatgtgg acctgccccc taaagagtca agcatctaa 1539

<210> 20

<211> 344

<212> PRT

<213> 人工序列

<220>

<223> 包含与Fc结构域融合的ActRIIB的可溶的细胞外结构域(A64;SEQ ID NO:17)的融合蛋白

<400> 20

Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala
1 5 10 15
Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu
20 25 30
Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser
35 40 45
Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe
50 55 60
Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln
65 70 75 80
Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr
85 90 95
His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro
100 105 110
Thr Ala Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala
115 120 125
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
130 135 140
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
145 150 155 160
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
165 170 175
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
180 185 190
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
195 200 205
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
210 215 220
Leu Pro Val Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
225 230 235 240
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr

245 250 255

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
260 265 270

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
275 280 285

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
290 295 300

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
305 310 315 320

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
325 330 335

Ser Leu Ser Leu Ser Pro Gly Lys
340

<210> 21

<211> 329

<212> PRT

<213> 人工序列

<220>

<223> 包含与Fc结构域融合的、带有C-末端15个氨基酸删除的(SEQ ID NO:18) ActRIIB的可溶的细胞外结构域(A64)的融合蛋白

<400> 21

Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala
1 5 10 15

Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu
20 25 30

Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser
35 40 45

Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe
50 55 60

Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln
65 70 75 80

Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr
85 90 95

His Leu Pro Glu Ala Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro
100 105 110

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
115 120 125

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
130 135 140

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
145 150 155 160
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
165 170 175
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
180 185 190
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
195 200 205
Ala Leu Pro Val Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
210 215 220
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
225 230 235 240
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
245 250 255
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
260 265 270
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
275 280 285
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
290 295 300
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
305 310 315 320
Lys Ser Leu Ser Leu Ser Pro Gly Lys
325

<210> 22
<211> 105
<212> PRT
<213> 人工序列
<220>
<223> 人ActRIIB可溶的(细胞外的),EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端5个氨基酸删除(SEQ ID NO:28的氨基酸25-129)且具有L79D突变的加工的多肽序列
<400> 22
Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15
Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45
Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln

50 55 60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95
Gly Pro Glu Val Thr Tyr Glu Pro Pro
100 105

<210> 23
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> 人ActRIIB可溶的(细胞外的),EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端3个氨基酸删除(SEQ ID NO:28的氨基酸25-131)、具有L79D突变的加工的多肽序列
<400> 23

Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15
Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45
Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60
Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105

<210> 24
<211> 360
<212> PRT
<213> 人工序列
<220>
<223> EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端3个氨基酸删除(SEQ ID NO:28的氨基酸25-131)、具有L79D突变并具有TPA前导序列的未加工的ActRIIB-Fc融合蛋白
<400> 24

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

1 5 10 15
Ala Val Phe Val Ser Pro Gly Ala Ala Glu Thr Arg Glu Cys Ile Tyr
20 25 30
Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu
35 40 45
Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp
50 55 60
Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp
65 70 75 80
Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu
85 90 95
Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu
100 105 110
Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu
115 120 125
Pro Pro Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala
130 135 140
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
145 150 155 160
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
165 170 175
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
180 185 190
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
195 200 205
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
210 215 220
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
225 230 235 240
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
245 250 255
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
260 265 270
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
275 280 285
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
290 295 300
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
305 310 315 320

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
325 330 335
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
340 345 350
Ser Leu Ser Leu Ser Pro Gly Lys
355 360

<210> 25
<211> 335
<212> PRT
<213> 人工序列
<220>
<223> EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端3个氨基酸删除(SEQ ID NO:28的氨基酸25-131)、具有L79D突变的加工的ActRIIB-Fc融合蛋白
<400> 25
Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15
Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45
Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60
Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Gly Gly Gly Thr His
100 105 110
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
115 120 125
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
130 135 140
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
145 150 155 160
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
165 170 175
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
180 185 190
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys

195 200 205

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
210 215 220

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
225 230 235 240

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
245 250 255

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
260 265 270

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
275 280 285

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
290 295 300

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
305 310 315 320

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330 335

<210> 26

<211> 115

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),加工的多肽序列(SEQ ID NO:16的氨基酸20-134)

<400> 26

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105 110

Ala Pro Thr
115

<210> 27

<211> 100

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),C-末端15个氨基酸删除的加工的多肽序列(SEQ ID NO:16的氨基酸20-119)

<400> 27

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15
Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30
Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser Ser
35 40 45
Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
50 55 60
Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80
Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95
Leu Pro Glu Ala
100

<210> 28

<211> 512

<212> PRT

<213> 智人

<220>

<223> 人ActRIIB前体蛋白序列(R64)

<400> 28

Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Trp
1 5 10 15
Pro Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
20 25 30
Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
35 40 45
Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
50 55 60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65 70 75 80
Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
85 90 95
Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
100 105 110
Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
115 120 125
Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
130 135 140
Pro Ile Gly Gly Leu Ser Leu Ile Val Leu Leu Ala Phe Trp Met Tyr
145 150 155 160
Arg His Arg Lys Pro Pro Tyr Gly His Val Asp Ile His Glu Asp Pro
165 170 175
Gly Pro Pro Pro Pro Ser Pro Leu Val Gly Leu Lys Pro Leu Gln Leu
180 185 190
Leu Glu Ile Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln
195 200 205
Leu Met Asn Asp Phe Val Ala Val Lys Ile Phe Pro Leu Gln Asp Lys
210 215 220
Gln Ser Trp Gln Ser Glu Arg Glu Ile Phe Ser Thr Pro Gly Met Lys
225 230 235 240
His Glu Asn Leu Leu Gln Phe Ile Ala Ala Glu Lys Arg Gly Ser Asn
245 250 255
Leu Glu Val Glu Leu Trp Leu Ile Thr Ala Phe His Asp Lys Gly Ser
260 265 270
Leu Thr Asp Tyr Leu Lys Gly Asn Ile Ile Thr Trp Asn Glu Leu Cys
275 280 285
His Val Ala Glu Thr Met Ser Arg Gly Leu Ser Tyr Leu His Glu Asp
290 295 300
Val Pro Trp Cys Arg Gly Glu Gly His Lys Pro Ser Ile Ala His Arg
305 310 315 320
Asp Phe Lys Ser Lys Asn Val Leu Leu Lys Ser Asp Leu Thr Ala Val
325 330 335
Leu Ala Asp Phe Gly Leu Ala Val Arg Phe Glu Pro Gly Lys Pro Pro
340 345 350
Gly Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu
355 360 365
Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe Leu Arg Ile

370 375 380

Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Val Ser Arg Cys
385 390 395 400

Lys Ala Ala Asp Gly Pro Val Asp Glu Tyr Met Leu Pro Phe Glu Glu
405 410 415

Glu Ile Gly Gln His Pro Ser Leu Glu Glu Leu Gln Glu Val Val Val
420 425 430

His Lys Lys Met Arg Pro Thr Ile Lys Asp His Trp Leu Lys His Pro
435 440 445

Gly Leu Ala Gln Leu Cys Val Thr Ile Glu Glu Cys Trp Asp His Asp
450 455 460

Ala Glu Ala Arg Leu Ser Ala Gly Cys Val Glu Glu Arg Val Ser Leu
465 470 475 480

Ile Arg Arg Ser Val Asn Gly Thr Thr Ser Asp Cys Leu Val Ser Leu
485 490 495

Val Thr Ser Val Thr Asn Val Asp Leu Pro Pro Lys Glu Ser Ser Ile
500 505 510

<210> 29

<211> 116

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),加工的多肽序列(SEQ ID NO:28的氨基酸19-134)

<400> 29

Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala
1 5 10 15

Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu
20 25 30

Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser
35 40 45

Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe
50 55 60

Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln
65 70 75 80

Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr
85 90 95

His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro
100 105 110

Thr Ala Pro Thr
115

<210> 30

<211> 101

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),C-末端15个氨基酸删除的加工的多肽序列(SEQ ID NO:28的氨基酸19-119)

<400> 30

Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala
1 5 10 15
Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu
20 25 30
Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser
35 40 45
Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe
50 55 60
Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln
65 70 75 80
Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr
85 90 95
His Leu Pro Glu Ala
100

<210> 31

<211> 115

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),加工的多肽序列(SEQ ID NO:28的氨基酸20-134)

<400> 31

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15
Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30
Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45
Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn

50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80
Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95
Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105 110
Ala Pro Thr
115

<210> 32
<211> 100
<212> PRT
<213> 人工序列
<220>
<223> 人ActRIIB可溶的(细胞外的),C-末端15个氨基酸删除的加工的多肽序列(SEQ ID NO:28的氨基酸20-119)
<400> 32
Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15
Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30
Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45
Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
50 55 60
Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80
Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95
Leu Pro Glu Ala
100

<210> 33
<211> 107
<212> PRT
<213> 人工序列
<220>
<223> 人ActRIIB可溶的(细胞外的),EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端3个氨基酸删除(SEQ ID NO:16的氨基酸25-131)、具有L79D突变的加工的多肽序列
<400> 33

Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15
Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30
Leu His Cys Tyr Ala Ser Trp Ala Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45
Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60
Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105

<210> 34

<211> 360

<212> PRT

<213> 人工序列

<220>

<223> EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端3个氨基酸删除(SEQ ID NO:16的氨基酸25-131)、具有L79D突变并具有TPA前导序列的未加工的ActRIIB-Fc融合蛋白

<400> 34

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15
Ala Val Phe Val Ser Pro Gly Ala Ala Glu Thr Arg Glu Cys Ile Tyr
20 25 30
Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu
35 40 45
Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp
50 55 60
Ala Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp
65 70 75 80
Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu
85 90 95
Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu
100 105 110
Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu
115 120 125

Pro Pro Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala
130 135 140
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
145 150 155 160
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
165 170 175
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
180 185 190
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
195 200 205
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
210 215 220
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
225 230 235 240
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
245 250 255
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
260 265 270
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
275 280 285
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
290 295 300
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
305 310 315 320
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
325 330 335
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
340 345 350
Ser Leu Ser Leu Ser Pro Gly Lys
355 360

<210> 35
<211> 335
<212> PRT
<213> 人工序列
<220>
<223> EC结构域的N-末端6个氨基酸删除、EC结构域的C-末端3个氨基酸删除(SEQ ID NO:16的氨基酸25-131)、具有L79D突变的加工的ActRIIB-Fc融合蛋白
<400> 35
Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg

1 5 10 15

Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30

Leu His Cys Tyr Ala Ser Trp Ala Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45

Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Gly Gly Gly Thr His
100 105 110

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
115 120 125

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
130 135 140

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
145 150 155 160

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
165 170 175

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
180 185 190

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
195 200 205

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
210 215 220

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
225 230 235 240

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
245 250 255

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
260 265 270

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
275 280 285

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
290 295 300

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
305 310 315 320

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330 335

<210> 36

<211> 115

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),具有L79D突变的加工的多肽序列(SEQ ID NO:28的氨基酸20-134)

<400> 36

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15
Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30
Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45
Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
50 55 60
Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80
Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95
Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105 110
Ala Pro Thr
115

<210> 37

<211> 115

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),具有L79D突变的加工的多肽序列(SEQ ID NO:16的氨基酸20-134)

<400> 37

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15
Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30
Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser Ser

35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105 110

Ala Pro Thr
115

<210> 38

<211> 343

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),具有通过GGG接头与Fc结构域融合的L79D突变的加工的多肽序列(SEQ ID NO:28的氨基酸20-134)

<400> 38

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105 110

Ala Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala Pro
115 120 125

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
130 135 140

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
145 150 155 160

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
165 170 175
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
180 185 190
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
195 200 205
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
210 215 220
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
225 230 235 240
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
245 250 255
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
260 265 270
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
275 280 285
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
290 295 300
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
305 310 315 320
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
325 330 335
Leu Ser Leu Ser Pro Gly Lys
340

<210> 39
<211> 343
<212> PRT
<213> 人工序列
<220>
<223> 人ActRIIB可溶的(细胞外的),具有与Fc结构域融合的L79D突变的加工的多肽序列(SEQ ID NO:16的氨基酸20-134)
<400> 39
Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15
Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30
Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser Ser
35 40 45
Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn

50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105 110

Ala Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala Pro
115 120 125

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
130 135 140

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
145 150 155 160

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
165 170 175

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
180 185 190

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
195 200 205

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
210 215 220

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
225 230 235 240

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
245 250 255

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
260 265 270

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
275 280 285

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
290 295 300

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
305 310 315 320

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
325 330 335

Leu Ser Leu Ser Pro Gly Lys
340

<210> 40

<211> 368

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),具有与Fc结构域融合的L79D突变和TPA前导序列的加工的多肽序列(SEQ ID NO:28的氨基酸20-134)

<400> 40

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15
Ala Val Phe Val Ser Pro Gly Ala Ser Gly Arg Gly Glu Ala Glu Thr
20 25 30
Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn
35 40 45
Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His
50 55 60
Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys
65 70 75 80
Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys
85 90 95
Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly
100 105 110
Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro
115 120 125
Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly Gly Thr
130 135 140
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
145 150 155 160
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
165 170 175
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
180 185 190
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
195 200 205
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
210 215 220
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
225 230 235 240
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
245 250 255
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu

260 265 270

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys

275 280 285

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser

290 295 300

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp

305 310 315 320

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser

325 330 335

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala

340 345 350

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

355 360 365

<210> 41

<211> 368

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),具有与Fc结构域融合的L79D突变和TPA前导序列的加工的多肽序列(SEQ ID NO:16的氨基酸20-134)

<400> 41

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

1 5 10 15

Ala Val Phe Val Ser Pro Gly Ala Ser Gly Arg Gly Glu Ala Glu Thr

20 25 30

Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn

35 40 45

Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His

50 55 60

Cys Tyr Ala Ser Trp Ala Asn Ser Ser Gly Thr Ile Glu Leu Val Lys

65 70 75 80

Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys

85 90 95

Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly

100 105 110

Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro

115 120 125

Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly Gly Thr

130 135 140

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
145 150 155 160
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
165 170 175
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
180 185 190
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
195 200 205
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
210 215 220
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
225 230 235 240
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
245 250 255
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
260 265 270
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
275 280 285
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
290 295 300
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
305 310 315 320
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
325 330 335
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
340 345 350
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
355 360 365

<210> 42
<211> 141
<212> PRT
<213> 人工序列
<220>
<223> 人ActRIIB可溶的(细胞外的),具有变体C-末端序列的加工的多肽序列(WO2007/053775中公开的)

<400> 42
Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15
Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly

20 25 30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Gly Pro Trp Ala Ser Thr Thr Ile
100 105 110

Pro Ser Gly Gly Pro Glu Ala Thr Ala Ala Ala Gly Asp Gln Gly Ser
115 120 125

Gly Ala Leu Trp Leu Cys Leu Glu Gly Pro Ala His Glu
130 135 140

<210> 43

<211> 141

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),具有带L79D突变的变体C-末端序列的加工的多肽序列(WO2007/053775中公开的)

<400> 43

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Gly Pro Trp Ala Ser Thr Thr Ile
100 105 110

Pro Ser Gly Gly Pro Glu Ala Thr Ala Ala Ala Gly Asp Gln Gly Ser
115 120 125

Gly Ala Leu Trp Leu Cys Leu Glu Gly Pro Ala His Glu
130 135 140

<210> 44

<211> 370

<212> PRT

<213> 人工序列

<220>

<223> 人ActRIIB可溶的(细胞外的),具有用TGGG接头融合到Fc结构域的L79D突变的变体C-末端序列的加工的多肽序列(WO2007/053775中公开的)

<400> 44

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Gly Pro Trp Ala Ser Thr Thr Ile
100 105 110

Pro Ser Gly Gly Pro Glu Ala Thr Ala Ala Ala Gly Asp Gln Gly Ser
115 120 125

Gly Ala Leu Trp Leu Cys Leu Glu Gly Pro Ala His Glu Thr Gly Gly
130 135 140

Gly Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
145 150 155 160

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
165 170 175

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
180 185 190

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
195 200 205

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
210 215 220

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys

225 230 235 240
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
245 250 255
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
260 265 270
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
275 280 285
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
290 295 300
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
305 310 315 320
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
325 330 335
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
340 345 350
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
355 360 365
Gly Lys
370

<210> 45
<211> 1083
<212> DNA
<213> 人工序列
<220>
<223> 编码SEQ ID NO:24的核酸序列
<400> 45
atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt 60
tcgcccggcg ccgccgaaac ccgcgaatgt atttattaca atgctaattg ggaactcgaa 120
cggacgaacc aatccgggct cgaacggtgt gaggggaac aggataaacg cctccattgc 180
tatgcgtcgt ggaggaactc ctccgggacg attgaactgg tcaagaaagg gtgctgggac 240
gacgatttca attgttatga ccgccaggaa tgtgtcgcga ccgaagagaa tccgcaggtc 300
tatttctgtt gttgcgaggg gaatttctgt aatgaacggt ttacccacct ccccgaagcc 360
ggcgggcccg aggtgaccta tgaacccccg cccaccggtg gtggaactca cacatgccca 420
ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc 480
aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 540
cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 600
aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 660
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 720
ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 780

gtgtacaccc tgccccatc ccgggaggag atgaccaaga accaggtcag cctgacctgc 840
ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 900
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctat 960
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1020
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc cccgggtaaa 1080
tga 1083

<210> 46
<211> 344
<212> PRT
<213> 人工序列
<220>
<223> 包含与Fc结构域融合的ActRIIB的可溶的细胞外结构域(R64;SEQ ID NO:29)的融合蛋白

<400> 46

Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala
1 5 10 15
Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu
20 25 30
Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser
35 40 45
Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe
50 55 60
Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln
65 70 75 80
Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr
85 90 95
His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro
100 105 110
Thr Ala Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala
115 120 125
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
130 135 140
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
145 150 155 160
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
165 170 175
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
180 185 190
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln

195 200 205

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
210 215 220

Leu Pro Val Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
225 230 235 240

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
245 250 255

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
260 265 270

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
275 280 285

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
290 295 300

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
305 310 315 320

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
325 330 335

Ser Leu Ser Leu Ser Pro Gly Lys
340

<210> 47

<211> 329

<212> PRT

<213> 人工序列

<220>

<223> 包含与Fc结构域融合的、带有C-末端15个氨基酸删除的(SEQ ID NO:30) ActRIIB的可溶的细胞外结构域(R64)的融合蛋白

<400> 47

Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala
1 5 10 15

Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu
20 25 30

Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser
35 40 45

Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe
50 55 60

Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln
65 70 75 80

Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr
85 90 95

His Leu Pro Glu Ala Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro
100 105 110
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
115 120 125
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
130 135 140
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
145 150 155 160
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
165 170 175
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
180 185 190
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
195 200 205
Ala Leu Pro Val Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
210 215 220
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
225 230 235 240
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
245 250 255
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
260 265 270
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
275 280 285
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
290 295 300
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
305 310 315 320
Lys Ser Leu Ser Leu Ser Pro Gly Lys
325

<210> 48
<211> 20
<212> DNA
<213> 人工序列
<220>
<223> Runx2 qRT-PCR引物1
<400> 48
ccgcacgaca accgcaccat 20
<210> 49

<211> 20
<212> DNA
<213> 人工序列
<220>
<223> Runx2 qRT-PCR引物2
<400> 49
cgctccggcc cacaaatctc 20
<210> 50
<211> 20
<212> DNA
<213> 人工序列
<220>
<223> Alp qRT-PCR引物1
<400> 50
acgtggctaa gaatgtcatc 20
<210> 51
<211> 19
<212> DNA
<213> 人工序列
<220>
<223> Alp qRT-PCR引物2
<400> 51
ctggtaggcg atgtcctta 19
<210> 52
<211> 25
<212> DNA
<213> 人工序列
<220>
<223> osterix qRT-PCR引物1
<400> 52
tagtggtttg gggtttgttt accgc 25
<210> 53
<211> 26
<212> DNA
<213> 人工序列
<220>
<223> osterix qRT-PCR引物2
<400> 53
aaccaaataa ctcttattcc ctaagt 26

<210> 54
<211> 21
<212> DNA
<213> 人工序列
<220>
<223> Klotho qRT-PCR引物1
<400> 54
gctctcaaag cccacatact g 21
<210> 55
<211> 20
<212> DNA
<213> 人工序列
<220>
<223> Klotho qRT-PCR引物1
<400> 55
gcagcataac gatagaggcc 20
<210> 56
<211> 22
<212> DNA
<213> 人工序列
<220>
<223> Sm22-alpha qRT-PCR引物1
<400> 56
gttccagact gttgacctct tt 22
<210> 57
<211> 20
<212> DNA
<213> 人工序列
<220>
<223> Sm22-alpha qRT-PCR引物2
<400> 57
ctgcgctttc ttcataaacc 20
<210> 58
<211> 5553
<212> DNA
<213> 智人
<220>
<223> 人Runx2 mRNA
<400> 58

gtgtgaatgc ttcattcgcc tcacaaacaa ccacagaacc acaagtgcgg tgcaaacttt 60
ctccaggagg acagcaagaa gtctctggtt tttaaatggt taatctccgc aggtcactac 120
cagccaccga gaccaacaga gtcatttaag gctgcaagca gtatttacaa cagagggtac 180
aagttctatc tgaaaaaaaa aggagggact atggcatcaa acagcctctt cagcacagtg 240
acaccatgtc agcaaaactt cttttgggat ccgagcacca gccggcgctt cagccccccc 300
tccagcagcc tgcagcccgg caaaatgagc gacgtgagcc cggtggtggc tgcgcaacag 360
cagcagcaac agcagcagca gcaacagcag cagcagcagc agcaacagca gcagcagcag 420
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ccccggttgc ggccgcccca cgacaaccgc accatggtgg agatcatcgc cgaccacccg 540
gccgaactcg tccgcaccga cagccccaac ttcctgtgct cggtgctgcc ctcgcactgg 600
cgctgcaaca agaccctgcc cgtggccttc aaggtggtag ccctcggaga ggtaccagat 660
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gcctctgctg ttatgaaaaa ccaagtagca aggttcaacg atctgagatt tgtgggccgg 780
agtggacgag gcaagagttt caccttgacc ataaccgtct tcacaaatcc tccccaagta 840
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agacagaagc ttgatgactc taaacctagt ttgttctctg accgcctcag tgatttaggg 960
cgcattcctc atcccagtat gagagtaggt gtcccgcctc agaacccacg gccctccctg 1020
aactctgcac caagtccttt taatccacaa ggacagagtc agattacaga ccccaggcag 1080
gcacagtctt ccccgccgtg gtcctatgac cagtcttacc cctcctacct gagccagatg 1140
acgtccccgt ccatccactc taccaccccg ctgtcttcca cacggggcac tgggcttcct 1200
gccatcaccg atgtgcctag gcgcatttca gatgatgaca ctgccacctc tgacttctgc 1260
ctctggcctt ccactctcag taagaagagc caggcaggtg cttcagaact gggccctttt 1320
tcagacccca ggcagttccc aagcatttca tccctcactg agagccgctt ctccaaccca 1380
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caaagccaga gtggaccctt ccagaccagc agcactccat atctctacta tggcacttcg 1560
tcaggatcct atcagtttcc catggtgccg gggggagacc ggtctccttc cagaatgctt 1620
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aatgatggtg ttgacgctga tggaagccac agcagttccc caactgtttt gaattctagt 1740
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tatctggggg ccacatccca cacgtatcaa tatatacata tatagagaga gtgcatatat 1860
atgtatatcg attagctatc tacaaagtgc ctatttttta gaagattttt cattcactca 1920
ctcagtcatg atcttgcagc cataagaggg tagatattga gaagcagaag gctcaagaga 1980
gacaattgca atcgagcttc agattgttta ctatttaaga tgtactttta caaaggaaca 2040
aagaagggaa aaggtatttt tgtttttgtt gtttggtctg ttatcatcaa taacctgttc 2100
atatgccaat tcagagaggt ggactccagg ttcaggaggg agaagagcaa agccgcttcc 2160
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cgcagacagc tcacaaaacc agttgaggtg cactaaaggg acatgaggta gaatggatgc 2280
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gacataaaaa tgctaccgcc agtgccagct gcatcctatt taattaaaaa ggtactatat 4200
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aatttgcact gggtcatgtg tttgatttgt gatttcaaga ccaaagcaaa gtcttactac 4560
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ctcacaaatt ttaacccata tcagagttcc agaacaggta ccacagcttt ggttttagat 4740
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ttagacggtc tcactgcctc tcacttgcca gagcccttc aaaatgagca gagaagtcca 4860
caccattagg gaccatctgt gataaattca gaagggagga gatgtgtgta cagctttaag 4920
gattccctca attccgagga aagggactgg cccagaatcc aggttaatac atggaaacac 4980
gaagcattag caaaagtaat aattatacct atggtatttg aaagaacaat aataaaagac 5040
acttcttcca aaccttgaat ttgttgtttt tagaaaacga atgcatttaa aaatattttc 5100
tatgtgagaa ttttttagat gtgtgtttac ttcatgttta caaataactg tttgcttttt 5160
aatgcagtac tttgaaatat atcagccaaa accataactt acaataattt cttaggtatt 5220
ctgaataaaa ttccatttct tttggatatg ctttaccatt cttaggtttc tgtggaacaa 5280
aaatatttgt agcattttgt gtaaatacaa gctttcattt ttatttttc caattgctat 5340
tgcccaagaa ttgctttcca tgcacatatt gtaaaaattc cgctttgtgc cacaggtcat 5400
gattgtggat gagtttactc ttaacttcaa agggactatt tgtattgtat gttgcaactg 5460
taaattgaat tatttggcat ttttctcatg attgtaatat taatttgaag tttgaattta 5520
attttcaata aaatggcttt tttggttttg tta 5553

<210> 59
<211> 3173
<212> DNA
<213> 智人
<220>
<223> 人Alp mRNA
<400> 59

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caaggactct ggagtcagag taggactgta ggaccggagc ctgagtggaa caggagtgga 120
gctggcctgg gagagagcgg atccctccca gcaccctcag gccacccgtt gcctgcactc 180
tccctgccag acctccagag aggagagact cgggacagcc agccccaggt tcccccagct 240
ctctccatct gcctggctcc ttgggacccg ttccccagcc tcaggatggc gtcctccctg 300
cttgaggagg aagttcacta tggctccagt cccctggcca tgctgacggc agcgtgcagc 360
aaatttggtg gctctagccc tctgcgggac tcaacaactc tgggcaaagc aggcacaaag 420
aagccgtact ctgtgggcag tgacctttca gcctccaaaa ccatggggga tgcttatcca 480
gcccccttta caagcactaa tgggctcctt tcacctgcag gcagtcctcc agcacccacc 540
tcaggctatg ctaatgatta ccctcccttt tcccactcat tccctgggcc cacaggcacc 600
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ggcatttcac caggcccagg caacactcct actccatggt gggatatgca ccctggaggc 780
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Ser Pro Ser Arg Met Leu Pro Pro Cys Thr Thr Thr Ser Asn Gly Ser
465 470 475 480

Thr Leu Leu Asn Pro Asn Leu Pro Asn Gln Asn Asp Gly Val Asp Ala
485 490 495

Asp Gly Ser His Ser Ser Ser Pro Thr Val Leu Asn Ser Ser Gly Arg
500 505 510

Met Asp Glu Ser Val Trp Arg Pro Tyr
515 520

<210> 64
<211> 524
<212> PRT
<213> 智人
<220>
<223> 人Alp蛋白
<400> 64

Met Ile Ser Pro Phe Leu Val Leu Ala Ile Gly Thr Cys Leu Thr Asn
1 5 10 15

Ser Leu Val Pro Glu Lys Glu Lys Asp Pro Lys Tyr Trp Arg Asp Gln
20 25 30

Ala Gln Glu Thr Leu Lys Tyr Ala Leu Glu Leu Gln Lys Leu Asn Thr
35 40 45

Asn Val Ala Lys Asn Val Ile Met Phe Leu Gly Asp Gly Met Gly Val
50 55 60

Ser Thr Val Thr Ala Ala Arg Ile Leu Lys Gly Gln Leu His His Asn
65 70 75 80

Pro Gly Glu Glu Thr Arg Leu Glu Met Asp Lys Phe Pro Phe Val Ala

85 90 95

Leu Ser Lys Thr Tyr Asn Thr Asn Ala Gln Val Pro Asp Ser Ala Gly
100 105 110

Thr Ala Thr Ala Tyr Leu Cys Gly Val Lys Ala Asn Glu Gly Thr Val
115 120 125

Gly Val Ser Ala Ala Thr Glu Arg Ser Arg Cys Asn Thr Thr Gln Gly
130 135 140

Asn Glu Val Thr Ser Ile Leu Arg Trp Ala Lys Asp Ala Gly Lys Ser
145 150 155 160

Val Gly Ile Val Thr Thr Thr Arg Val Asn His Ala Thr Pro Ser Ala
165 170 175

Ala Tyr Ala His Ser Ala Asp Arg Asp Trp Tyr Ser Asp Asn Glu Met
180 185 190

Pro Pro Glu Ala Leu Ser Gln Gly Cys Lys Asp Ile Ala Tyr Gln Leu
195 200 205

Met His Asn Ile Arg Asp Ile Asp Val Ile Met Gly Gly Gly Arg Lys
210 215 220

Tyr Met Tyr Pro Lys Asn Lys Thr Asp Val Glu Tyr Glu Ser Asp Glu
225 230 235 240

Lys Ala Arg Gly Thr Arg Leu Asp Gly Leu Asp Leu Val Asp Thr Trp
245 250 255

Lys Ser Phe Lys Pro Arg Tyr Lys His Ser His Phe Ile Trp Asn Arg
260 265 270

Thr Glu Leu Leu Thr Leu Asp Pro His Asn Val Asp Tyr Leu Leu Gly
275 280 285

Leu Phe Glu Pro Gly Asp Met Gln Tyr Glu Leu Asn Arg Asn Asn Val
290 295 300

Thr Asp Pro Ser Leu Ser Glu Met Val Val Val Ala Ile Gln Ile Leu
305 310 315 320

Arg Lys Asn Pro Lys Gly Phe Phe Leu Leu Val Glu Gly Gly Arg Ile
325 330 335

Asp His Gly His His Glu Gly Lys Ala Lys Gln Ala Leu His Glu Ala
340 345 350

Val Glu Met Asp Arg Ala Ile Gly Gln Ala Gly Ser Leu Thr Ser Ser
355 360 365

Glu Asp Thr Leu Thr Val Val Thr Ala Asp His Ser His Val Phe Thr
370 375 380

Phe Gly Gly Tyr Thr Pro Arg Gly Asn Ser Ile Phe Gly Leu Ala Pro
385 390 395 400

Met Leu Ser Asp Thr Asp Lys Lys Pro Phe Thr Ala Ile Leu Tyr Gly
405 410 415
Asn Gly Pro Gly Tyr Lys Val Val Gly Gly Glu Arg Glu Asn Val Ser
420 425 430
Met Val Asp Tyr Ala His Asn Asn Tyr Gln Ala Gln Ser Ala Val Pro
435 440 445
Leu Arg His Glu Thr His Gly Gly Glu Asp Val Ala Val Phe Ser Lys
450 455 460
Gly Pro Met Ala His Leu Leu His Gly Val His Glu Gln Asn Tyr Val
465 470 475 480
Pro His Val Met Ala Tyr Ala Ala Cys Ile Gly Ala Asn Leu Gly His
485 490 495
Cys Ala Pro Ala Ser Ser Ala Gly Ser Leu Ala Ala Gly Pro Leu Leu
500 505 510
Leu Ala Leu Ala Leu Tyr Pro Leu Ser Val Leu Phe
515 520

<210> 65
<211> 431
<212> PRT
<213> 智人
<220>
<223> 人Osterix蛋白
<400> 65

Met Ala Ser Ser Leu Leu Glu Glu Glu Val His Tyr Gly Ser Ser Pro
1 5 10 15
Leu Ala Met Leu Thr Ala Ala Cys Ser Lys Phe Gly Gly Ser Ser Pro
20 25 30
Leu Arg Asp Ser Thr Thr Leu Gly Lys Ala Gly Thr Lys Lys Pro Tyr
35 40 45
Ser Val Gly Ser Asp Leu Ser Ala Ser Lys Thr Met Gly Asp Ala Tyr
50 55 60
Pro Ala Pro Phe Thr Ser Thr Asn Gly Leu Leu Ser Pro Ala Gly Ser
65 70 75 80
Pro Pro Ala Pro Thr Ser Gly Tyr Ala Asn Asp Tyr Pro Pro Phe Ser
85 90 95
His Ser Phe Pro Gly Pro Thr Gly Thr Gln Asp Pro Gly Leu Leu Val
100 105 110
Pro Lys Gly His Ser Ser Ser Asp Cys Leu Pro Ser Val Tyr Thr Ser
115 120 125

Leu Asp Met Thr His Pro Tyr Gly Ser Trp Tyr Lys Ala Gly Ile His
130 135 140
Ala Gly Ile Ser Pro Gly Pro Gly Asn Thr Pro Thr Pro Trp Trp Asp
145 150 155 160
Met His Pro Gly Gly Asn Trp Leu Gly Gly Gly Gln Gly Gln Gly Asp
165 170 175
Gly Leu Gln Gly Thr Leu Pro Thr Gly Pro Ala Gln Pro Pro Leu Asn
180 185 190
Pro Gln Leu Pro Thr Tyr Pro Ser Asp Phe Ala Pro Leu Asn Pro Ala
195 200 205
Pro Tyr Pro Ala Pro His Leu Leu Gln Pro Gly Pro Gln His Val Leu
210 215 220
Pro Gln Asp Val Tyr Lys Pro Lys Ala Val Gly Asn Ser Gly Gln Leu
225 230 235 240
Glu Gly Ser Gly Gly Ala Lys Pro Pro Arg Gly Ala Ser Thr Gly Gly
245 250 255
Ser Gly Gly Tyr Gly Gly Ser Gly Ala Gly Arg Ser Ser Cys Asp Cys
260 265 270
Pro Asn Cys Gln Glu Leu Glu Arg Leu Gly Ala Ala Ala Ala Gly Leu
275 280 285
Arg Lys Lys Pro Ile His Ser Cys His Ile Pro Gly Cys Gly Lys Val
290 295 300
Tyr Gly Lys Ala Ser His Leu Lys Ala His Leu Arg Trp His Thr Gly
305 310 315 320
Glu Arg Pro Phe Val Cys Asn Trp Leu Phe Cys Gly Lys Arg Phe Thr
325 330 335
Arg Ser Asp Glu Leu Glu Arg His Val Arg Thr His Thr Arg Glu Lys
340 345 350
Lys Phe Thr Cys Leu Leu Cys Ser Lys Arg Phe Thr Arg Ser Asp His
355 360 365
Leu Ser Lys His Gln Arg Thr His Gly Glu Pro Gly Pro Gly Pro Pro
370 375 380
Pro Ser Gly Pro Lys Glu Leu Gly Glu Gly Arg Ser Thr Gly Glu Glu
385 390 395 400
Glu Ala Ser Gln Thr Pro Arg Pro Ser Ala Ser Pro Ala Thr Pro Glu
405 410 415
Lys Ala Pro Gly Gly Ser Pro Glu Gln Ser Asn Leu Leu Glu Ile
420 425 430

<210> 66

<211> 1012
<212> PRT
<213> 智人
<220>
<223> 人Klotho蛋白
<400> 66

Met Pro Ala Ser Ala Pro Pro Arg Arg Pro Arg Pro Pro Pro Pro Ser
1 5 10 15
Leu Ser Leu Leu Leu Val Leu Leu Gly Leu Gly Gly Arg Arg Leu Arg
20 25 30
Ala Glu Pro Gly Asp Gly Ala Gln Thr Trp Ala Arg Phe Ser Arg Pro
35 40 45
Pro Ala Pro Glu Ala Ala Gly Leu Phe Gln Gly Thr Phe Pro Asp Gly
50 55 60
Phe Leu Trp Ala Val Gly Ser Ala Ala Tyr Gln Thr Glu Gly Gly Trp
65 70 75 80
Gln Gln His Gly Lys Gly Ala Ser Ile Trp Asp Thr Phe Thr His His
85 90 95
Pro Leu Ala Pro Pro Gly Asp Ser Arg Asn Ala Ser Leu Pro Leu Gly
100 105 110
Ala Pro Ser Pro Leu Gln Pro Ala Thr Gly Asp Val Ala Ser Asp Ser
115 120 125
Tyr Asn Asn Val Phe Arg Asp Thr Glu Ala Leu Arg Glu Leu Gly Val
130 135 140
Thr His Tyr Arg Phe Ser Ile Ser Trp Ala Arg Val Leu Pro Asn Gly
145 150 155 160
Ser Ala Gly Val Pro Asn Arg Glu Gly Leu Arg Tyr Tyr Arg Arg Leu
165 170 175
Leu Glu Arg Leu Arg Glu Leu Gly Val Gln Pro Val Val Thr Leu Tyr
180 185 190
His Trp Asp Leu Pro Gln Arg Leu Gln Asp Ala Tyr Gly Gly Trp Ala
195 200 205
Asn Arg Ala Leu Ala Asp His Phe Arg Asp Tyr Ala Glu Leu Cys Phe
210 215 220
Arg His Phe Gly Gly Gln Val Lys Tyr Trp Ile Thr Ile Asp Asn Pro
225 230 235 240
Tyr Val Val Ala Trp His Gly Tyr Ala Thr Gly Arg Leu Ala Pro Gly
245 250 255
Ile Arg Gly Ser Pro Arg Leu Gly Tyr Leu Val Ala His Asn Leu Leu

260 265 270

Leu Ala His Ala Lys Val Trp His Leu Tyr Asn Thr Ser Phe Arg Pro
275 280 285

Thr Gln Gly Gly Gln Val Ser Ile Ala Leu Ser Ser His Trp Ile Asn
290 295 300

Pro Arg Arg Met Thr Asp His Ser Ile Lys Glu Cys Gln Lys Ser Leu
305 310 315 320

Asp Phe Val Leu Gly Trp Phe Ala Lys Pro Val Phe Ile Asp Gly Asp
325 330 335

Tyr Pro Glu Ser Met Lys Asn Asn Leu Ser Ser Ile Leu Pro Asp Phe
340 345 350

Thr Glu Ser Glu Lys Lys Phe Ile Lys Gly Thr Ala Asp Phe Phe Ala
355 360 365

Leu Cys Phe Gly Pro Thr Leu Ser Phe Gln Leu Leu Asp Pro His Met
370 375 380

Lys Phe Arg Gln Leu Glu Ser Pro Asn Leu Arg Gln Leu Leu Ser Trp
385 390 395 400

Ile Asp Leu Glu Phe Asn His Pro Gln Ile Phe Ile Val Glu Asn Gly
405 410 415

Trp Phe Val Ser Gly Thr Thr Lys Arg Asp Asp Ala Lys Tyr Met Tyr
420 425 430

Tyr Leu Lys Lys Phe Ile Met Glu Thr Leu Lys Ala Ile Lys Leu Asp
435 440 445

Gly Val Asp Val Ile Gly Tyr Thr Ala Trp Ser Leu Met Asp Gly Phe
450 455 460

Glu Trp His Arg Gly Tyr Ser Ile Arg Arg Gly Leu Phe Tyr Val Asp
465 470 475 480

Phe Leu Ser Gln Asp Lys Met Leu Leu Pro Lys Ser Ser Ala Leu Phe
485 490 495

Tyr Gln Lys Leu Ile Glu Lys Asn Gly Phe Pro Pro Leu Pro Glu Asn
500 505 510

Gln Pro Leu Glu Gly Thr Phe Pro Cys Asp Phe Ala Trp Gly Val Val
515 520 525

Asp Asn Tyr Ile Gln Val Asp Thr Thr Leu Ser Gln Phe Thr Asp Leu
530 535 540

Asn Val Tyr Leu Trp Asp Val His His Ser Lys Arg Leu Ile Lys Val
545 550 555 560

Asp Gly Val Val Thr Lys Lys Arg Lys Ser Tyr Cys Val Asp Phe Ala
565 570 575

Ala Ile Gln Pro Gln Ile Ala Leu Leu Gln Glu Met His Val Thr His
580 585 590
Phe Arg Phe Ser Leu Asp Trp Ala Leu Ile Leu Pro Leu Gly Asn Gln
595 600 605
Ser Gln Val Asn His Thr Ile Leu Gln Tyr Tyr Arg Cys Met Ala Ser
610 615 620
Glu Leu Val Arg Val Asn Ile Thr Pro Val Val Ala Leu Trp Gln Pro
625 630 635 640
Met Ala Pro Asn Gln Gly Leu Pro Arg Leu Leu Ala Arg Gln Gly Ala
645 650 655
Trp Glu Asn Pro Tyr Thr Ala Leu Ala Phe Ala Glu Tyr Ala Arg Leu
660 665 670
Cys Phe Gln Glu Leu Gly His His Val Lys Leu Trp Ile Thr Met Asn
675 680 685
Glu Pro Tyr Thr Arg Asn Met Thr Tyr Ser Ala Gly His Asn Leu Leu
690 695 700
Lys Ala His Ala Leu Ala Trp His Val Tyr Asn Glu Lys Phe Arg His
705 710 715 720
Ala Gln Asn Gly Lys Ile Ser Ile Ala Leu Gln Ala Asp Trp Ile Glu
725 730 735
Pro Ala Cys Pro Phe Ser Gln Lys Asp Lys Glu Val Ala Glu Arg Val
740 745 750
Leu Glu Phe Asp Ile Gly Trp Leu Ala Glu Pro Ile Phe Gly Ser Gly
755 760 765
Asp Tyr Pro Trp Val Met Arg Asp Trp Leu Asn Gln Arg Asn Asn Phe
770 775 780
Leu Leu Pro Tyr Phe Thr Glu Asp Glu Lys Lys Leu Ile Gln Gly Thr
785 790 795 800
Phe Asp Phe Leu Ala Leu Ser His Tyr Thr Thr Ile Leu Val Asp Ser
805 810 815
Glu Lys Glu Asp Pro Ile Lys Tyr Asn Asp Tyr Leu Glu Val Gln Glu
820 825 830
Met Thr Asp Ile Thr Trp Leu Asn Ser Pro Ser Gln Val Ala Val Val
835 840 845
Pro Trp Gly Leu Arg Lys Val Leu Asn Trp Leu Lys Phe Lys Tyr Gly
850 855 860
Asp Leu Pro Met Tyr Ile Ile Ser Asn Gly Ile Asp Asp Gly Leu His
865 870 875 880
Ala Glu Asp Asp Gln Leu Arg Val Tyr Tyr Met Gln Asn Tyr Ile Asn

885 890 895

Glu Ala Leu Lys Ala His Ile Leu Asp Gly Ile Asn Leu Cys Gly Tyr
900 905 910

Phe Ala Tyr Ser Phe Asn Asp Arg Thr Ala Pro Arg Phe Gly Leu Tyr
915 920 925

Arg Tyr Ala Ala Asp Gln Phe Glu Pro Lys Ala Ser Met Lys His Tyr
930 935 940

Arg Lys Ile Ile Asp Ser Asn Gly Phe Pro Gly Pro Glu Thr Leu Glu
945 950 955 960

Arg Phe Cys Pro Glu Glu Phe Thr Val Cys Thr Glu Cys Ser Phe Phe
965 970 975

His Thr Arg Lys Ser Leu Leu Ala Phe Ile Ala Phe Leu Phe Phe Ala
980 985 990

Ser Ile Ile Ser Leu Ser Leu Ile Phe Tyr Tyr Ser Lys Lys Gly Arg
995 1000 1005

Arg Ser Tyr Lys
1010

<210> 67
<211> 201
<212> PRT
<213> 智人
<220>
<223> 人Sm22-alpha蛋白
<400> 67

Met Ala Asn Lys Gly Pro Ser Tyr Gly Met Ser Arg Glu Val Gln Ser
1 5 10 15

Lys Ile Glu Lys Lys Tyr Asp Glu Glu Leu Glu Glu Arg Leu Val Glu
20 25 30

Trp Ile Ile Val Gln Cys Gly Pro Asp Val Gly Arg Pro Asp Arg Gly
35 40 45

Arg Leu Gly Phe Gln Val Trp Leu Lys Asn Gly Val Ile Leu Ser Lys
50 55 60

Leu Val Asn Ser Leu Tyr Pro Asp Gly Ser Lys Pro Val Lys Val Pro
65 70 75 80

Glu Asn Pro Pro Ser Met Val Phe Lys Gln Met Glu Gln Val Ala Gln
85 90 95

Phe Leu Lys Ala Ala Glu Asp Tyr Gly Val Ile Lys Thr Asp Met Phe
100 105 110

Gln Thr Val Asp Leu Phe Glu Gly Lys Asp Met Ala Ala Val Gln Arg

115 120 125

Thr Leu Met Ala Leu Gly Ser Leu Ala Val Thr Lys Asn Asp Gly His
130 135 140

Tyr Arg Gly Asp Pro Asn Trp Phe Met Lys Lys Ala Gln Glu His Lys
145 150 155 160

Arg Glu Phe Thr Glu Ser Gln Leu Gln Glu Gly Lys His Val Ile Gly
165 170 175

Leu Gln Met Gly Ser Asn Arg Gly Ala Ser Gln Ala Gly Met Thr Gly
180 185 190

Tyr Gly Arg Pro Arg Gln Ile Ile Ser
195 200

<210> 68
<211> 377
<212> PRT
<213> 智人
<220>
<223> 人alpha-SMA蛋白
<400> 68

Met Cys Glu Glu Glu Asp Ser Thr Ala Leu Val Cys Asp Asn Gly Ser
1 5 10 15

Gly Leu Cys Lys Ala Gly Phe Ala Gly Asp Asp Ala Pro Arg Ala Val
20 25 30

Phe Pro Ser Ile Val Gly Arg Pro Arg His Gln Gly Val Met Val Gly
35 40 45

Met Gly Gln Lys Asp Ser Tyr Val Gly Asp Glu Ala Gln Ser Lys Arg
50 55 60

Gly Ile Leu Thr Leu Lys Tyr Pro Ile Glu His Gly Ile Ile Thr Asn
65 70 75 80

Trp Asp Asp Met Glu Lys Ile Trp His His Ser Phe Tyr Asn Glu Leu
85 90 95

Arg Val Ala Pro Glu Glu His Pro Thr Leu Leu Thr Glu Ala Pro Leu
100 105 110

Asn Pro Lys Ala Asn Arg Glu Lys Met Thr Gln Ile Met Phe Glu Thr
115 120 125

Phe Asn Val Pro Ala Met Tyr Val Ala Ile Gln Ala Val Leu Ser Leu
130 135 140

Tyr Ala Ser Gly Arg Thr Thr Gly Ile Val Leu Asp Ser Gly Asp Gly
145 150 155 160

Val Thr His Asn Val Pro Ile Tyr Glu Gly Tyr Ala Leu Pro His Ala

165 170 175

Ile Met Arg Leu Asp Leu Ala Gly Arg Asp Leu Thr Asp Tyr Leu Met
180 185 190

Lys Ile Leu Thr Glu Arg Gly Tyr Ser Phe Val Thr Thr Ala Glu Arg
195 200 205

Glu Ile Val Arg Asp Ile Lys Glu Lys Leu Cys Tyr Val Ala Leu Asp
210 215 220

Phe Glu Asn Glu Met Ala Thr Ala Ala Ser Ser Ser Ser Leu Glu Lys
225 230 235 240

Ser Tyr Glu Leu Pro Asp Gly Gln Val Ile Thr Ile Gly Asn Glu Arg
245 250 255

Phe Arg Cys Pro Glu Thr Leu Phe Gln Pro Ser Phe Ile Gly Met Glu
260 265 270

Ser Ala Gly Ile His Glu Thr Thr Tyr Asn Ser Ile Met Lys Cys Asp
275 280 285

Ile Asp Ile Arg Lys Asp Leu Tyr Ala Asn Asn Val Leu Ser Gly Gly
290 295 300

Thr Thr Met Tyr Pro Gly Ile Ala Asp Arg Met Gln Lys Glu Ile Thr
305 310 315 320

Ala Leu Ala Pro Ser Thr Met Lys Ile Lys Ile Ile Ala Pro Pro Glu
325 330 335

Arg Lys Tyr Ser Val Trp Ile Gly Gly Ser Ile Leu Ala Ser Leu Ser
340 345 350

Thr Phe Gln Gln Met Trp Ile Ser Lys Gln Glu Tyr Asp Glu Ala Gly
355 360 365

Pro Ser Ile Val His Arg Lys Cys Phe
370 375

<210> 69

<211> 986

<212> PRT

<213> 智人

<220>

<223> 人MYOCD蛋白

<400> 69

Met Thr Leu Leu Gly Ser Glu His Ser Leu Leu Ile Arg Ser Lys Phe
1 5 10 15

Arg Ser Val Leu Gln Leu Arg Leu Gln Gln Arg Arg Thr Gln Glu Gln
20 25 30

Leu Ala Asn Gln Gly Ile Ile Pro Pro Leu Lys Arg Pro Ala Glu Phe

35 40 45

His Glu Gln Arg Lys His Leu Asp Ser Asp Lys Ala Lys Asn Ser Leu
50 55 60

Lys Arg Lys Ala Arg Asn Arg Cys Asn Ser Ala Asp Leu Val Asn Met
65 70 75 80

His Ile Leu Gln Ala Ser Thr Ala Glu Arg Ser Ile Pro Thr Ala Gln
85 90 95

Met Lys Leu Lys Arg Ala Arg Leu Ala Asp Asp Leu Asn Glu Lys Ile
100 105 110

Ala Leu Arg Pro Gly Pro Leu Glu Leu Val Glu Lys Asn Ile Leu Pro
115 120 125

Val Asp Ser Ala Val Lys Glu Ala Ile Lys Gly Asn Gln Val Ser Phe
130 135 140

Ser Lys Ser Thr Asp Ala Phe Ala Phe Glu Glu Asp Ser Ser Ser Asp
145 150 155 160

Gly Leu Ser Pro Asp Gln Thr Arg Ser Glu Asp Pro Gln Asn Ser Ala
165 170 175

Gly Ser Pro Pro Asp Ala Lys Ala Ser Asp Thr Pro Ser Thr Gly Ser
180 185 190

Leu Gly Thr Asn Gln Asp Leu Ala Ser Gly Ser Glu Asn Asp Arg Asn
195 200 205

Asp Ser Ala Ser Gln Pro Ser His Gln Ser Asp Ala Gly Lys Gln Gly
210 215 220

Leu Gly Pro Pro Ser Thr Pro Ile Ala Val His Ala Ala Val Lys Ser
225 230 235 240

Lys Ser Leu Gly Asp Ser Lys Asn Arg His Lys Lys Pro Lys Asp Pro
245 250 255

Lys Pro Lys Val Lys Lys Leu Lys Tyr His Gln Tyr Ile Pro Pro Asp
260 265 270

Gln Lys Ala Glu Lys Ser Pro Pro Pro Met Asp Ser Ala Tyr Ala Arg
275 280 285

Leu Leu Gln Gln Gln Gln Leu Phe Leu Gln Leu Gln Ile Leu Ser Gln
290 295 300

Gln Gln Gln Gln Gln Gln His Arg Phe Ser Tyr Leu Gly Met His Gln
305 310 315 320

Ala Gln Leu Lys Glu Pro Asn Glu Gln Met Val Arg Asn Pro Asn Ser
325 330 335

Ser Ser Thr Pro Leu Ser Asn Thr Pro Leu Ser Pro Val Lys Asn Ser
340 345 350

Phe Ser Gly Gln Thr Gly Val Ser Ser Phe Lys Pro Gly Pro Leu Pro
355 360 365
Pro Asn Leu Asp Asp Leu Lys Val Ser Glu Leu Arg Gln Gln Leu Arg
370 375 380
Ile Arg Gly Leu Pro Val Ser Gly Thr Lys Thr Ala Leu Met Asp Arg
385 390 395 400
Leu Arg Pro Phe Gln Asp Cys Ser Gly Asn Pro Val Pro Asn Phe Gly
405 410 415
Asp Ile Thr Thr Val Thr Phe Pro Val Thr Pro Asn Thr Leu Pro Asn
420 425 430
Tyr Gln Ser Ser Ser Ser Thr Ser Ala Leu Ser Asn Gly Phe Tyr His
435 440 445
Phe Gly Ser Thr Ser Ser Ser Pro Pro Ile Ser Pro Ala Ser Ser Asp
450 455 460
Leu Ser Val Ala Gly Ser Leu Pro Asp Thr Phe Asn Asp Ala Ser Pro
465 470 475 480
Ser Phe Gly Leu His Pro Ser Pro Val His Val Cys Thr Glu Glu Ser
485 490 495
Leu Met Ser Ser Leu Asn Gly Gly Ser Val Pro Ser Glu Leu Asp Gly
500 505 510
Leu Asp Ser Glu Lys Asp Lys Met Leu Val Glu Lys Gln Lys Val Ile
515 520 525
Asn Glu Leu Thr Trp Lys Leu Gln Gln Glu Gln Arg Gln Val Glu Glu
530 535 540
Leu Arg Met Gln Leu Gln Lys Gln Lys Arg Asn Asn Cys Ser Glu Lys
545 550 555 560
Lys Pro Leu Pro Phe Leu Ala Ala Ser Ile Lys Gln Glu Glu Ala Val
565 570 575
Ser Ser Cys Pro Phe Ala Ser Gln Val Pro Val Lys Arg Gln Ser Ser
580 585 590
Ser Ser Glu Cys His Pro Pro Ala Cys Glu Ala Ala Gln Leu Gln Pro
595 600 605
Leu Gly Asn Ala His Cys Val Glu Ser Ser Asp Gln Thr Asn Val Leu
610 615 620
Ser Ser Thr Phe Leu Ser Pro Gln Cys Ser Pro Gln His Ser Pro Leu
625 630 635 640
Gly Ala Val Lys Ser Pro Gln His Ile Ser Leu Pro Pro Ser Pro Asn
645 650 655
Asn Pro His Phe Leu Pro Ser Ser Ser Gly Ala Gln Gly Glu Gly His

660 665 670

Arg Val Ser Ser Pro Ile Ser Ser Gln Val Cys Thr Ala Gln Asn Ser

675 680 685

Gly Ala His Asp Gly His Pro Pro Ser Phe Ser Pro His Ser Ser Ser

690 695 700

Leu His Pro Pro Phe Ser Gly Ala Gln Ala Asp Ser Ser His Gly Ala

705 710 715 720

Gly Gly Asn Pro Cys Pro Lys Ser Pro Cys Val Gln Gln Lys Met Ala

725 730 735

Gly Leu His Ser Ser Asp Lys Val Gly Pro Lys Phe Ser Ile Pro Ser

740 745 750

Pro Thr Phe Ser Lys Ser Ser Ser Ala Ile Ser Glu Val Thr Gln Pro

755 760 765

Pro Ser Tyr Glu Asp Ala Val Lys Gln Gln Met Thr Arg Ser Gln Gln

770 775 780

Met Asp Glu Leu Leu Asp Val Leu Ile Glu Ser Gly Glu Met Pro Ala

785 790 795 800

Asp Ala Arg Glu Asp His Ser Cys Leu Gln Lys Val Pro Lys Ile Pro

805 810 815

Arg Ser Ser Arg Ser Pro Thr Ala Val Leu Thr Lys Pro Ser Ala Ser

820 825 830

Phe Glu Gln Ala Ser Ser Gly Ser Gln Ile Pro Phe Asp Pro Tyr Ala

835 840 845

Thr Asp Ser Asp Glu His Leu Glu Val Leu Leu Asn Ser Gln Ser Pro

850 855 860

Leu Gly Lys Met Ser Asp Val Thr Leu Leu Lys Ile Gly Ser Glu Glu

865 870 875 880

Pro His Phe Asp Gly Ile Met Asp Gly Phe Ser Gly Lys Ala Ala Glu

885 890 895

Asp Leu Phe Asn Ala His Glu Ile Leu Pro Gly Pro Leu Ser Pro Met

900 905 910

Gln Thr Gln Phe Ser Pro Ser Ser Val Asp Ser Asn Gly Leu Gln Leu

915 920 925

Ser Phe Thr Glu Ser Pro Trp Glu Thr Met Glu Trp Leu Asp Leu Thr

930 935 940

Pro Pro Asn Ser Thr Pro Gly Phe Ser Ala Leu Thr Thr Ser Ser Pro

945 950 955 960

Ser Ile Phe Asn Ile Asp Phe Leu Asp Val Thr Asp Leu Asn Leu Asn

965 970 975

Ser Ser Met Asp Leu His Leu Gln Gln Trp
980 985

<210> 70
<211> 1798
<212> DNA
<213> 智人
<220>
<223> 人alpha-SMA mRNA
<400> 70

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tttccaaatc attcctagcc aaagctctga ctcgttacct atgtgttttt taataaatct 1740
gaaataggct actggtaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa 1798

<210> 71
<211> 8580
<212> DNA
<213> 智人
<220>
<223> 人MYOCD mRNA
<400> 71

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ggggaggcgc cagttttctg gggacactgg ctgccactgt actcctaccc aggggagctc 180
acggagagtt ggatgaattc tgggttgtta gctgcggtca gctgggctcc cgggagcctg 240
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tgacactcct ggggtctgag cattccttgc tgattaggag caagttcaga tcagttttac 360
agttaagact tcaacaaaga aggacccagg aacaactggc taaccaaggc ataataccac 420
cactgaaacg tccagctgaa ttccatgagc aaagaaaaca tttggatagt gacaaggcta 480
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cctagggatc tgaaatccat aaactaagag aaactgtgta tagcttacct gaacaggaat 3180
ccttactgat atttatagaa cagttgattt cccccatccc cagtttatgg atatgctgct 3240
ttaaacttgg aaggggggaga caggaagttt taattgttct gactaaactt aggagttgag 3300
ctaggagtgc gttcatggtt tcttcactaa cagaggaatt atgctttgca ctacgtccct 3360
ccaagtgaag acagactgtt ttagacagac tttttaaaat ggtgccctac cattgacaca 3420
tgcagaaatt ggtgcgtttt gtttttttt ttcctatgct gctctgtttt gtcttaaagg 3480
tcttgagggt tgaccatgtt gcgtcatcat caacattttg ggggttgtgt tggatgggat 3540
gatctgttgc agagggagag gcagggaacc ctgctccttc gggccccagg ttgatcctgt 3600
gactgaggct ccccctcatg tagcctcccc aggcccaggg ccctgaggcc tgctagaatc 3660
actgccgctg tgctttcgtg gaaatgacag ttccttgttt tttttgtttc tgtttttgtt 3720
ttacattagt cattggacca cagccattca ggaactaccc cctgccccac aaagaaatga 3780
acagttgtag ggagacccag cagcaccttt cctccacaca ccttcatttt gatgttcggg 3840
tttttgtgtt aagttaatct gtacattctg tttgccattg ttacttgtac tatacatctg 3900
tatatagtgt acggcaaaag agtattaatc cactatctct agtgcttgac tttaaatcag 3960
tacagtacct gtacctgcac ggtcacccgc tccgtgtgtc gccctatatt gagggctcaa 4020
gctttccctt gtttttgaa aggggtttat gtataaatat attttatgcc tttttattac 4080
aagtcttgta ctcaatgact tttgtcatga cattttgttc tacttatact gtaaattatg 4140
cattataaag agttcattta aggaaaatta cttggtacaa taattattgt aattaagaga 4200
tgtagccttt attaaaattt tatatttttc aaaaaaaaaa a 4241

<210> 87
<211> 843
<212> PRT
<213> 智人
<220>
<223> 人Axin2氨基酸序列
<400> 87

Met Ser Ser Ala Met Leu Val Thr Cys Leu Pro Asp Pro Ser Ser Ser
1 5 10 15
Phe Arg Glu Asp Ala Pro Arg Pro Pro Val Pro Gly Glu Glu Gly Glu
20 25 30
Thr Pro Pro Cys Gln Pro Gly Val Gly Lys Gly Gln Val Thr Lys Pro
35 40 45
Met Pro Val Ser Ser Asn Thr Arg Arg Asn Glu Asp Gly Leu Gly Glu
50 55 60
Pro Glu Gly Arg Ala Ser Pro Asp Ser Pro Leu Thr Arg Trp Thr Lys
65 70 75 80
Ser Leu His Ser Leu Leu Gly Asp Gln Asp Gly Ala Tyr Leu Phe Arg
85 90 95
Thr Phe Leu Glu Arg Glu Lys Cys Val Asp Thr Leu Asp Phe Trp Phe
100 105 110
Ala Cys Asn Gly Phe Arg Gln Met Asn Leu Lys Asp Thr Lys Thr Leu
115 120 125
Arg Val Ala Lys Ala Ile Tyr Lys Arg Tyr Ile Glu Asn Asn Ser Ile
130 135 140
Val Ser Lys Gln Leu Lys Pro Ala Thr Lys Thr Tyr Ile Arg Asp Gly
145 150 155 160
Ile Lys Lys Gln Gln Ile Asp Ser Ile Met Phe Asp Gln Ala Gln Thr
165 170 175
Glu Ile Gln Ser Val Met Glu Glu Asn Ala Tyr Gln Met Phe Leu Thr
180 185 190
Ser Asp Ile Tyr Leu Glu Tyr Val Arg Ser Gly Gly Glu Asn Thr Ala
195 200 205
Tyr Met Ser Asn Gly Gly Leu Gly Ser Leu Lys Val Val Cys Gly Tyr
210 215 220
Leu Pro Thr Leu Asn Glu Glu Glu Glu Trp Thr Cys Ala Asp Phe Lys
225 230 235 240
Cys Lys Leu Ser Pro Thr Val Val Gly Leu Ser Ser Lys Thr Leu Arg
245 250 255

Ala Thr Ala Ser Val Arg Ser Thr Glu Thr Val Asp Ser Gly Tyr Arg
260 265 270
Ser Phe Lys Arg Ser Asp Pro Val Asn Pro Tyr His Ile Gly Ser Gly
275 280 285
Tyr Val Phe Ala Pro Ala Thr Ser Ala Asn Asp Ser Glu Ile Ser Ser
290 295 300
Asp Ala Leu Thr Asp Asp Ser Met Ser Met Thr Asp Ser Ser Val Asp
305 310 315 320
Gly Ile Pro Pro Tyr Arg Val Gly Ser Lys Lys Gln Leu Gln Arg Glu
325 330 335
Met His Arg Ser Val Lys Ala Asn Gly Gln Val Ser Leu Pro His Phe
340 345 350
Pro Arg Thr His Arg Leu Pro Lys Glu Met Thr Pro Val Glu Pro Ala
355 360 365
Thr Phe Ala Ala Glu Leu Ile Ser Arg Leu Glu Lys Leu Lys Leu Glu
370 375 380
Leu Glu Ser Arg His Ser Leu Glu Glu Arg Leu Gln Gln Ile Arg Glu
385 390 395 400
Asp Glu Glu Arg Glu Gly Ser Glu Leu Thr Leu Asn Ser Arg Glu Gly
405 410 415
Ala Pro Thr Gln His Pro Leu Ser Leu Leu Pro Ser Gly Ser Tyr Glu
420 425 430
Glu Asp Pro Gln Thr Ile Leu Asp Asp His Leu Ser Arg Val Leu Lys
435 440 445
Thr Pro Gly Cys Gln Ser Pro Gly Val Gly Arg Tyr Ser Pro Arg Ser
450 455 460
Arg Ser Pro Asp His His His His His His Ser Gln Tyr His Ser Leu
465 470 475 480
Leu Pro Pro Gly Gly Lys Leu Pro Pro Ala Ala Ala Ser Pro Gly Ala
485 490 495
Cys Pro Leu Leu Gly Gly Lys Gly Phe Val Thr Lys Gln Thr Thr Lys
500 505 510
His Val His His His Tyr Ile His His His Ala Val Pro Lys Thr Lys
515 520 525
Glu Glu Ile Glu Ala Glu Ala Thr Gln Arg Val His Cys Phe Cys Pro
530 535 540
Gly Gly Ser Glu Tyr Tyr Cys Tyr Ser Lys Cys Lys Ser His Ser Lys
545 550 555 560
Ala Pro Glu Thr Met Pro Ser Glu Gln Phe Gly Gly Ser Arg Gly Ser

565 570 575

Thr Leu Pro Lys Arg Asn Gly Lys Gly Thr Glu Pro Gly Leu Ala Leu

580 585 590

Pro Ala Arg Glu Gly Gly Ala Pro Gly Gly Ala Gly Ala Leu Gln Leu

595 600 605

Pro Arg Glu Glu Gly Asp Arg Ser Gln Asp Val Trp Gln Trp Met Leu

610 615 620

Glu Ser Glu Arg Gln Ser Lys Pro Lys Pro His Ser Ala Gln Ser Thr

625 630 635 640

Lys Lys Ala Tyr Pro Leu Glu Ser Ala Arg Ser Ser Pro Gly Glu Arg

645 650 655

Ala Ser Arg His His Leu Trp Gly Gly Asn Ser Gly His Pro Arg Thr

660 665 670

Thr Pro Arg Ala His Leu Phe Thr Gln Asp Pro Ala Met Pro Pro Leu

675 680 685

Thr Pro Pro Asn Thr Leu Ala Gln Leu Glu Glu Ala Cys Arg Arg Leu

690 695 700

Ala Glu Val Ser Lys Pro Pro Lys Gln Arg Cys Cys Val Ala Ser Gln

705 710 715 720

Gln Arg Asp Arg Asn His Ser Ala Thr Val Gln Thr Gly Ala Thr Pro

725 730 735

Phe Ser Asn Pro Ser Leu Ala Pro Glu Asp His Lys Glu Pro Lys Lys

740 745 750

Leu Ala Gly Val His Ala Leu Gln Ala Ser Glu Leu Val Val Thr Tyr

755 760 765

Phe Phe Cys Gly Glu Glu Ile Pro Tyr Arg Arg Met Leu Lys Ala Gln

770 775 780

Ser Leu Thr Leu Gly His Phe Lys Glu Gln Leu Ser Lys Lys Gly Asn

785 790 795 800

Tyr Arg Tyr Tyr Phe Lys Lys Ala Ser Asp Glu Phe Ala Cys Gly Ala

805 810 815

Val Phe Glu Glu Ile Trp Glu Asp Glu Thr Val Leu Pro Met Tyr Glu

820 825 830

Gly Arg Ile Leu Gly Lys Val Glu Arg Ile Asp

835 840

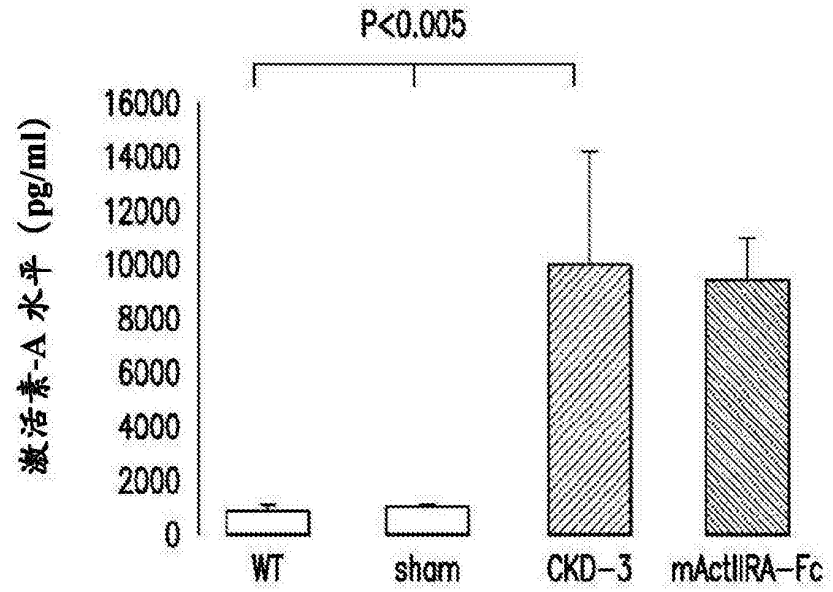

P<0.005
激活素-A 水平（pg/ml）
16000
14000
12000
10000
8000
6000
4000
2000
0
WT
sham
CKD-3
mActIIRA-Fc

图1A

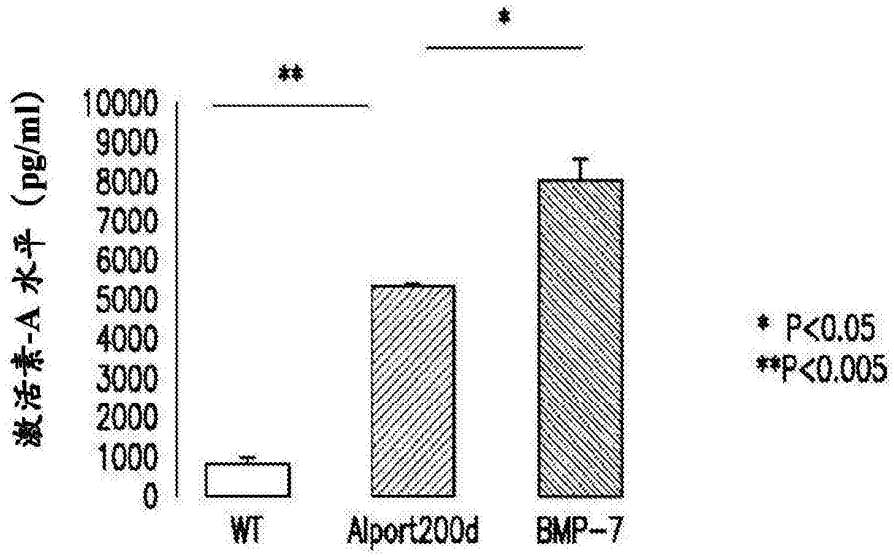

*
**
激活素-A 水平（pg/ml）
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
WT
Alport200d
BMP-7
* P<0.05
**P<0.005

图1B

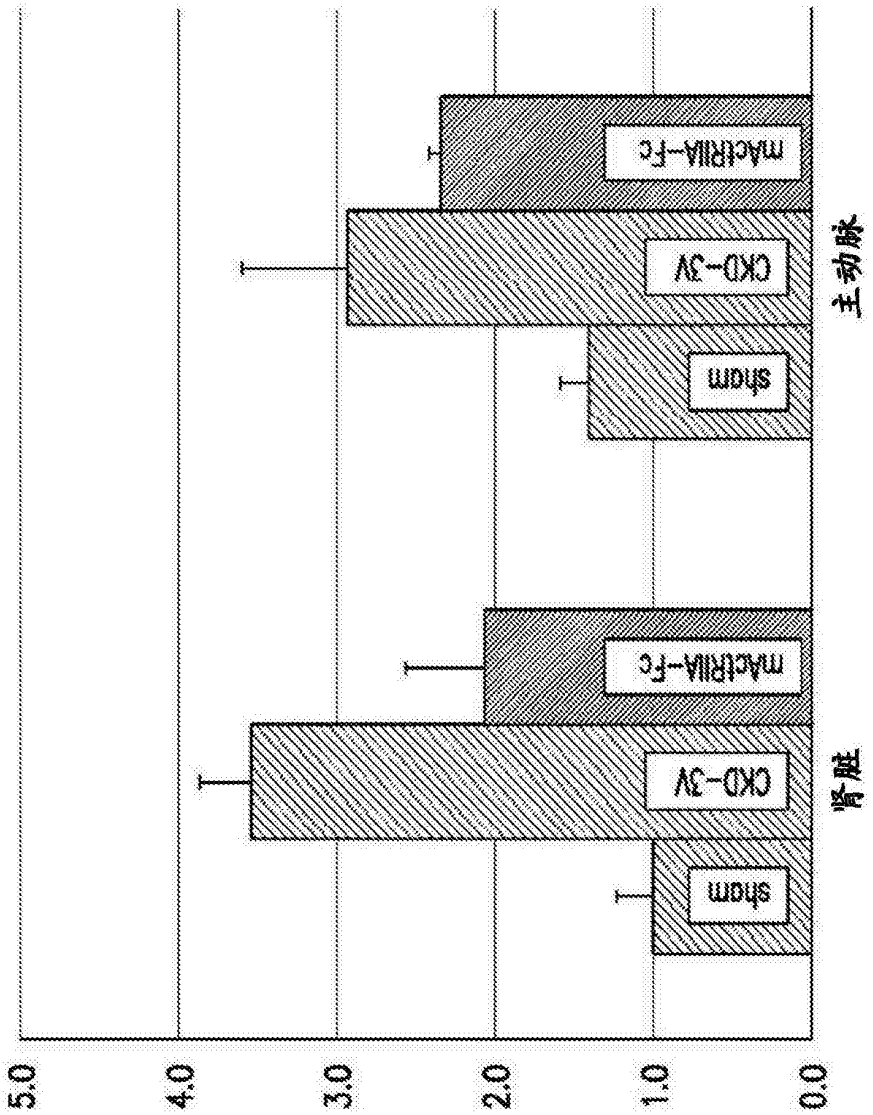

mActRIIA-Fc
CKD-3V
sham
主动脉
mActRIIA-Fc
CKD-3V
sham
肾脏
5.0
4.0
3.0
2.0
1.0
0.0
相对于 Sham 肾脏归一化到 B2m 的抑制素 betaA mRNA 表达

图2

图 3B

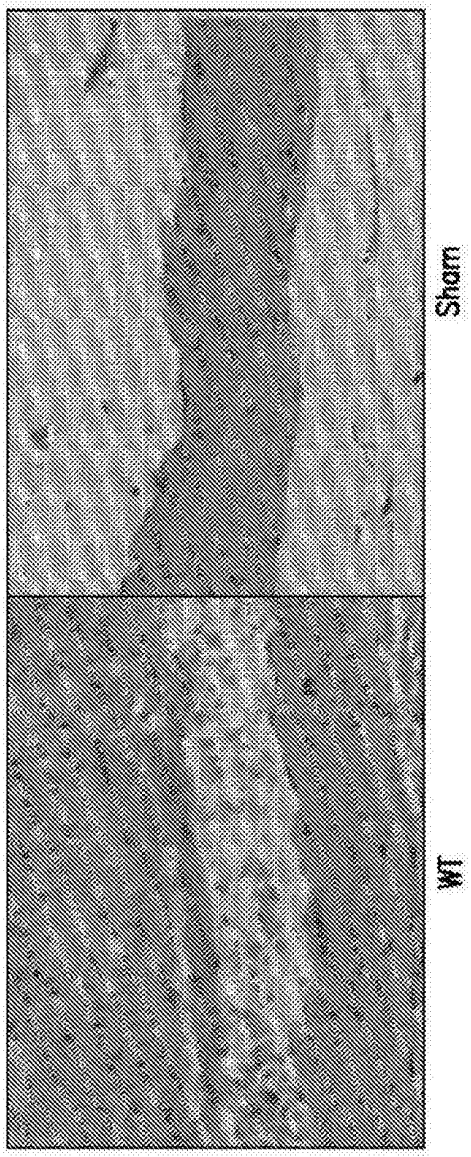


图 3A

图 3D

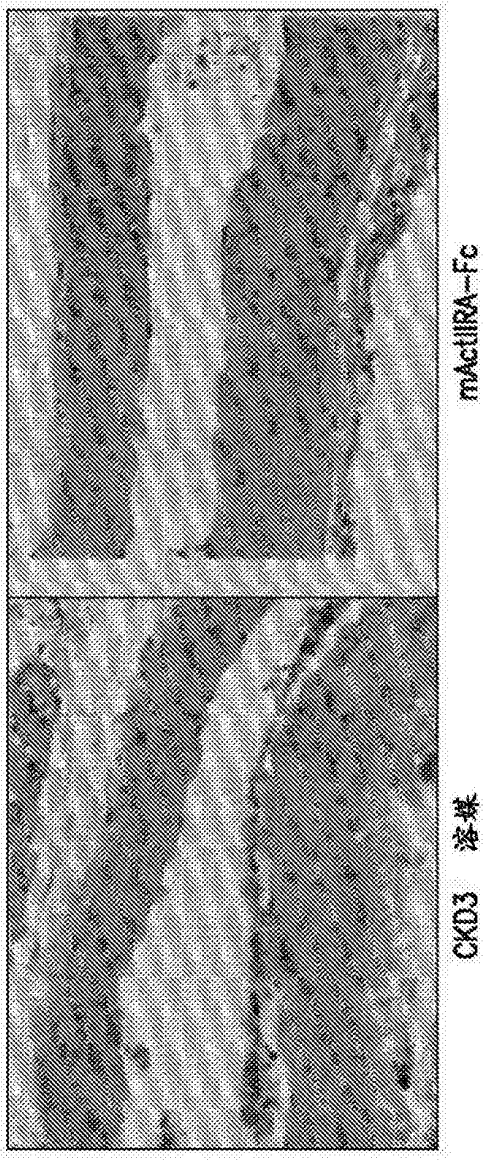


图 3C

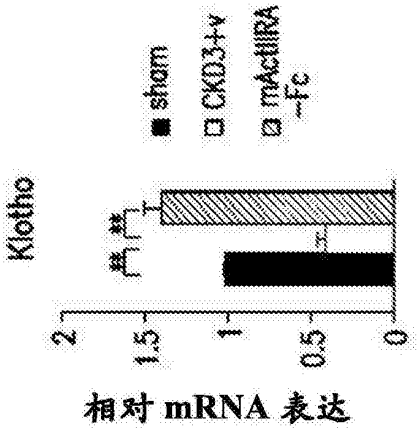


图 4C

*p<0.05
**p<0.01
***p<0.005

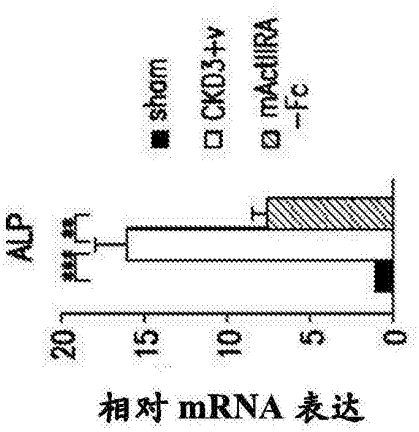


图 4B

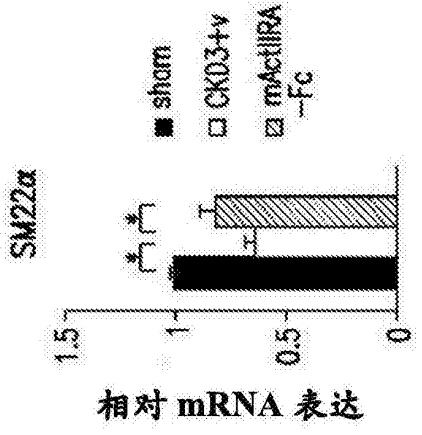


图 4E

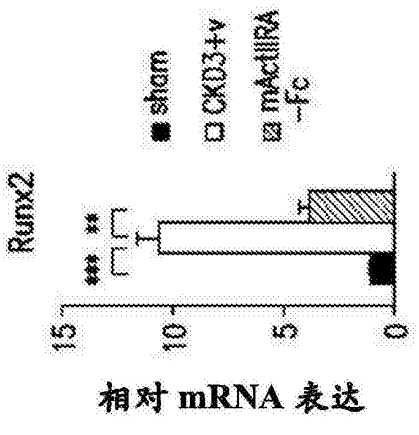


图 4A

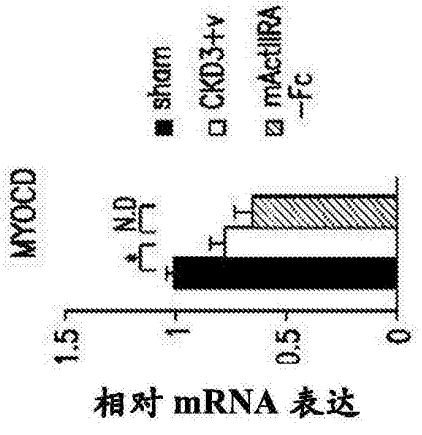


图 4D

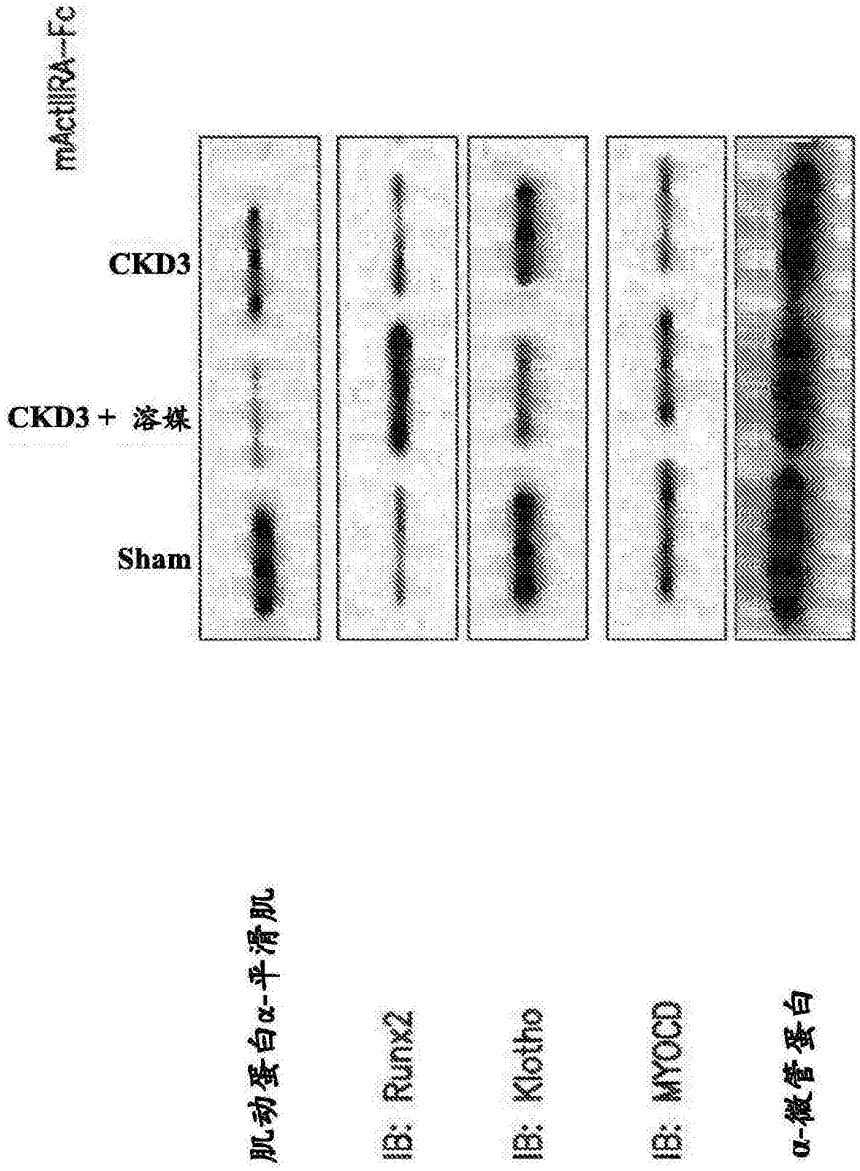

mActIIRA-Fc
CKD3
CKD3 + 溶媒
Sham
肌动蛋白α-平滑肌
IB: Runx2
IB: Klotho
IB: MYOCD
α-微管蛋白

图4F



图5A

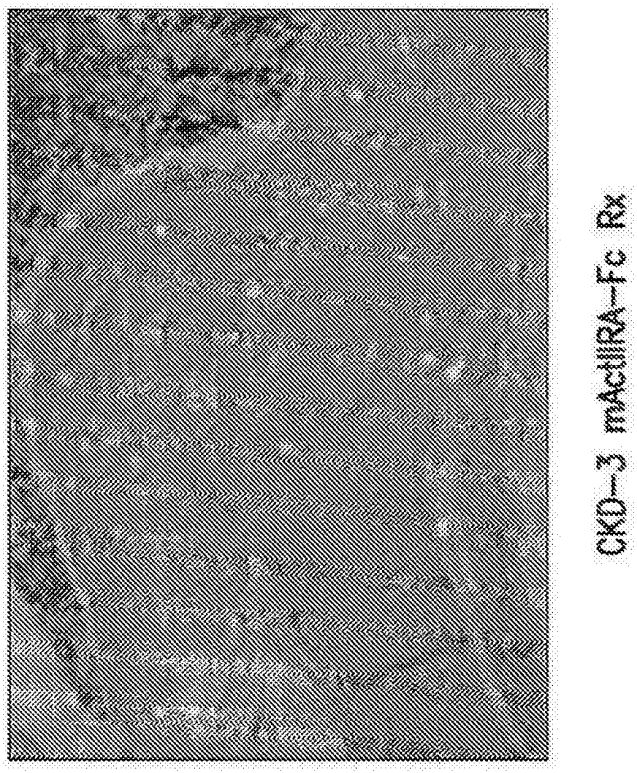

CKD-3 mActIIRA-Fc Rx

图5B

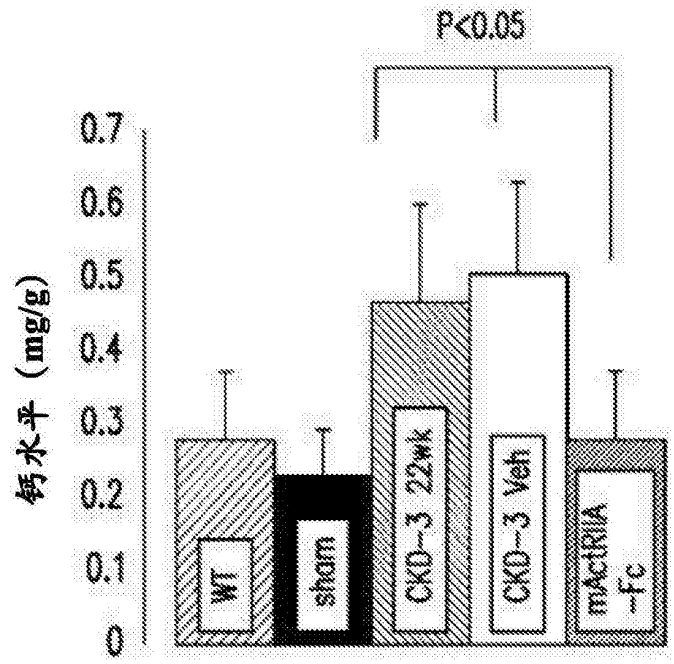

P<0.05
钙水平（mg/g）
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WT
sham
CKD-3 22wk
CKD-3 Veh
mActRIIA -Fc

图6A

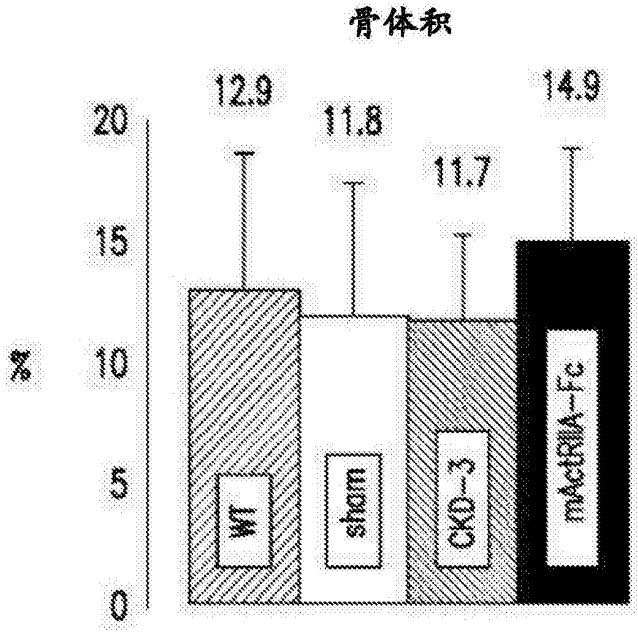
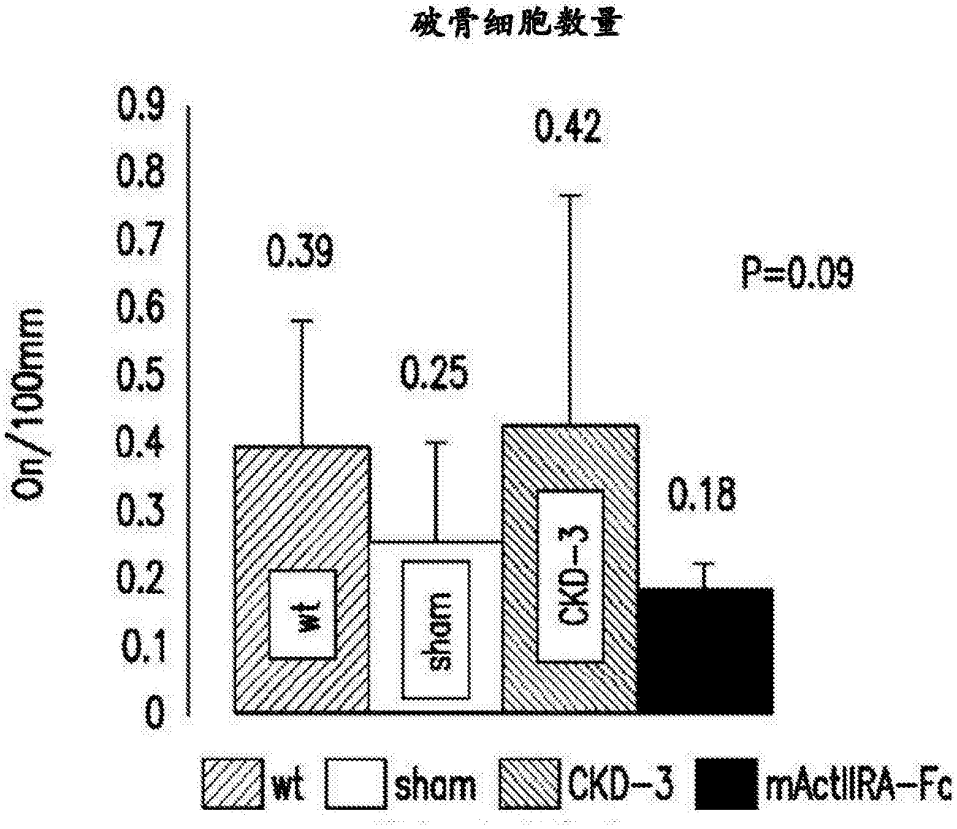

骨体积
12.9
11.8
11.7
14.9
20
15
10
5
0
%
WT
sham
CKD-3
mActRIIA-Fc

图6B


破骨细胞数量
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
On/100mm
0.39
0.25
0.42
0.18
P=0.09
wt
sham
CKD-3
wt
sham
CKD-3
mActIIRA-Fc

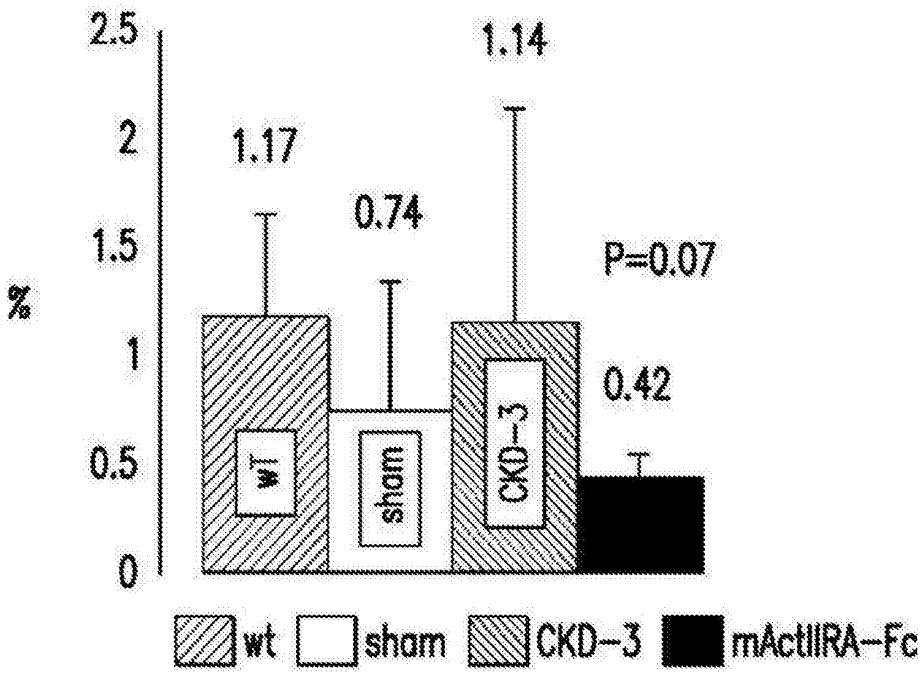

破骨细胞表面
2.5
2
1.5
1
0.5
0
%
1.17
0.74
1.14
P=0.07
0.42
wt
sham
CKD-3
wt
sham
CKD-3
mActIIRA-Fc

图6D

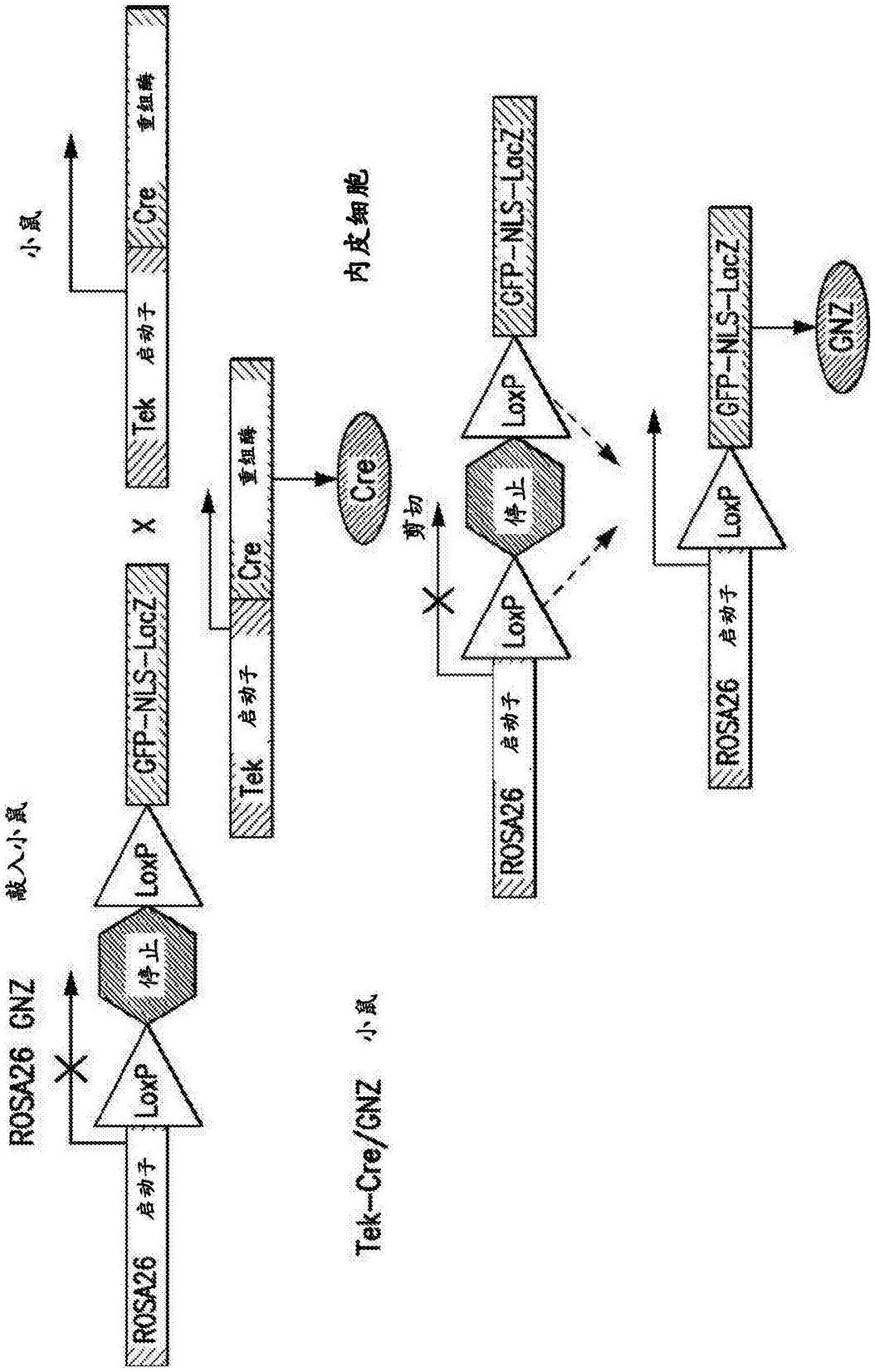

小鼠
Tek 启动子 Cre 重组酶
X
GFP-NLS-LacZ
LoxP
停止
LoxP
ROSA26 启动子
ROSA26 GNZ
敲入小鼠
Tek 启动子 Cre 重组酶
Cre
内皮细胞
剪切
GFP-NLS-LacZ
LoxP
停止
LoxP
ROSA26 启动子
GFP-NLS-LacZ
LoxP
ROSA26 启动子
GNZ
Tek-Cre/GNZ 小鼠

图7A

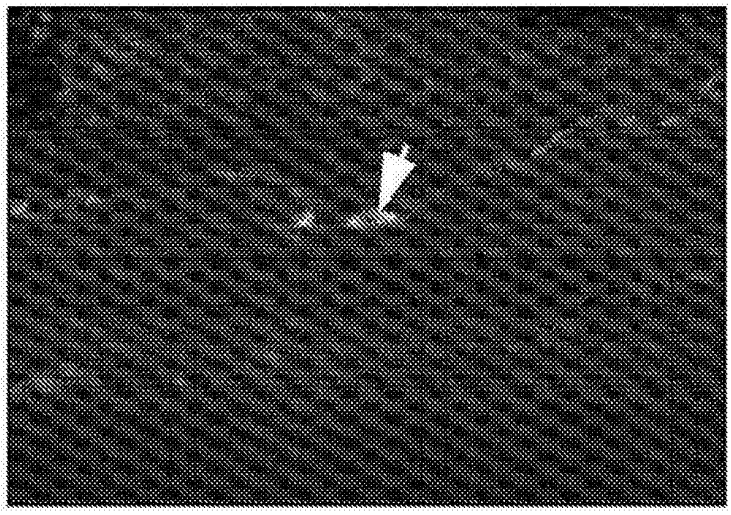


图7B

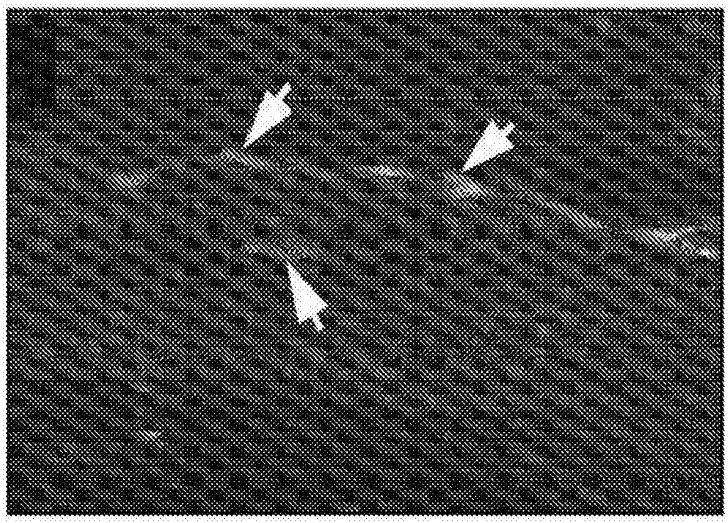


图7C

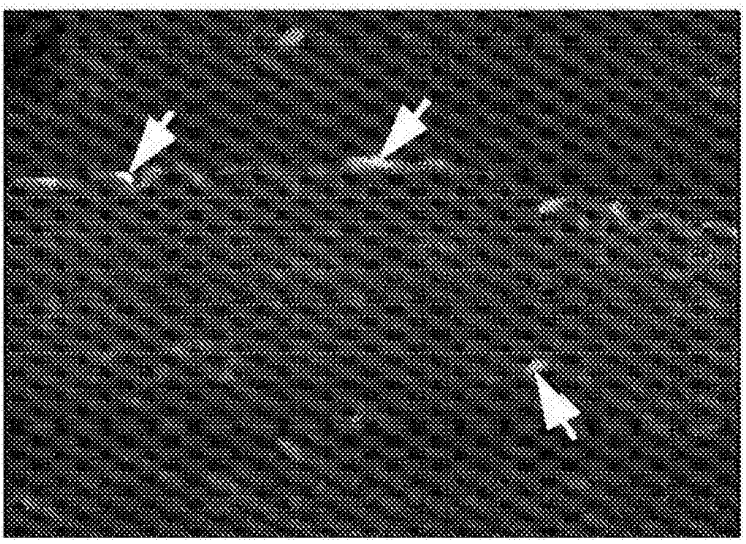


图7D

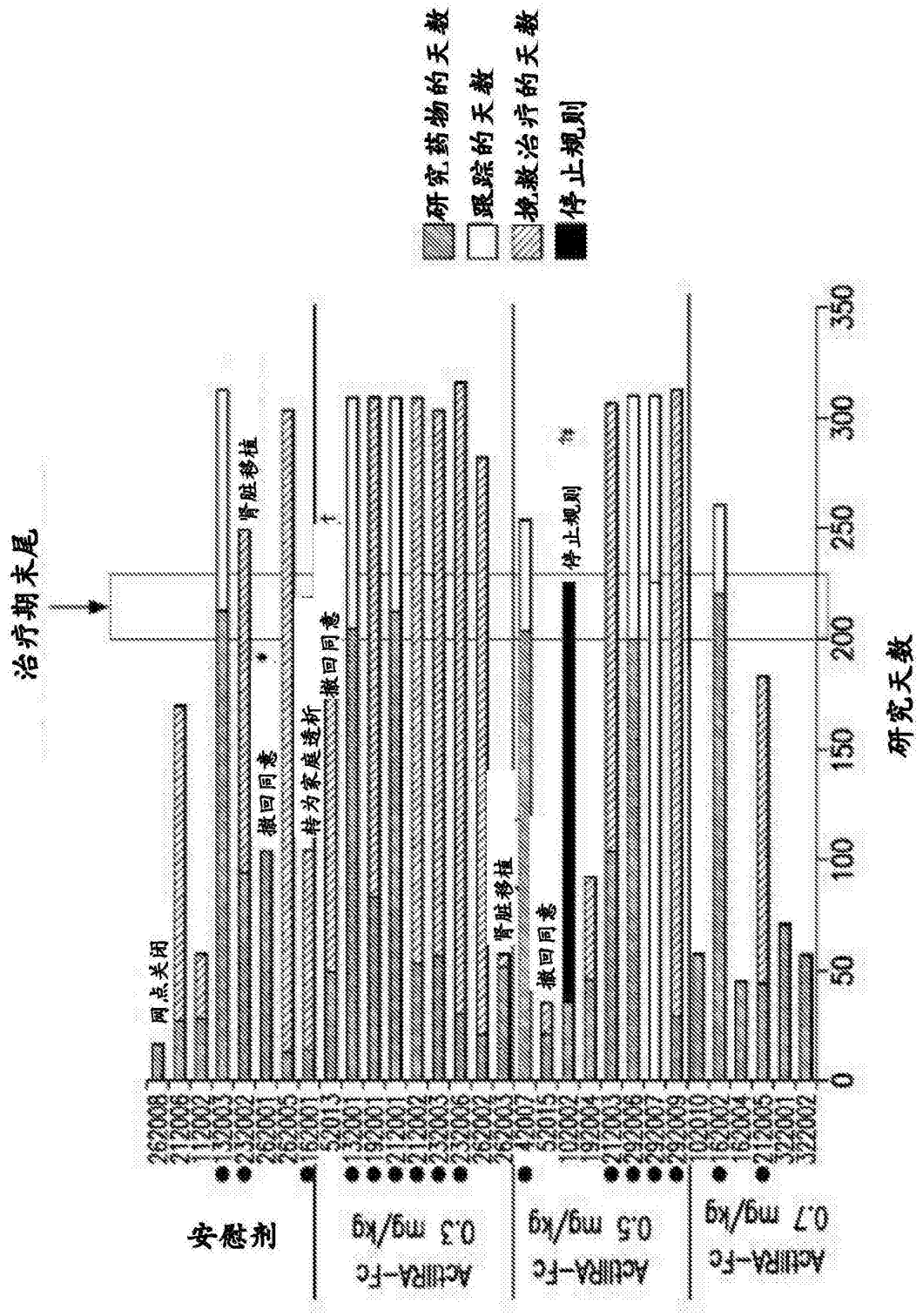

研究药物的天数
跟踪的天数
挽救治疗的天数
停止规则
治疗期末尾
网点关闭
撤回同意
肾脏移植
转为家庭透析
撤回同意
肾脏移植
撤回同意
停止规则
安慰剂
ACUIRA-Fc 0.3 mg/kg
ACUIRA-Fc 0.5 mg/kg
ACUIRA-Fc 0.7 mg/kg
研究天数
0
50
100
150
200
250
300
350

图8

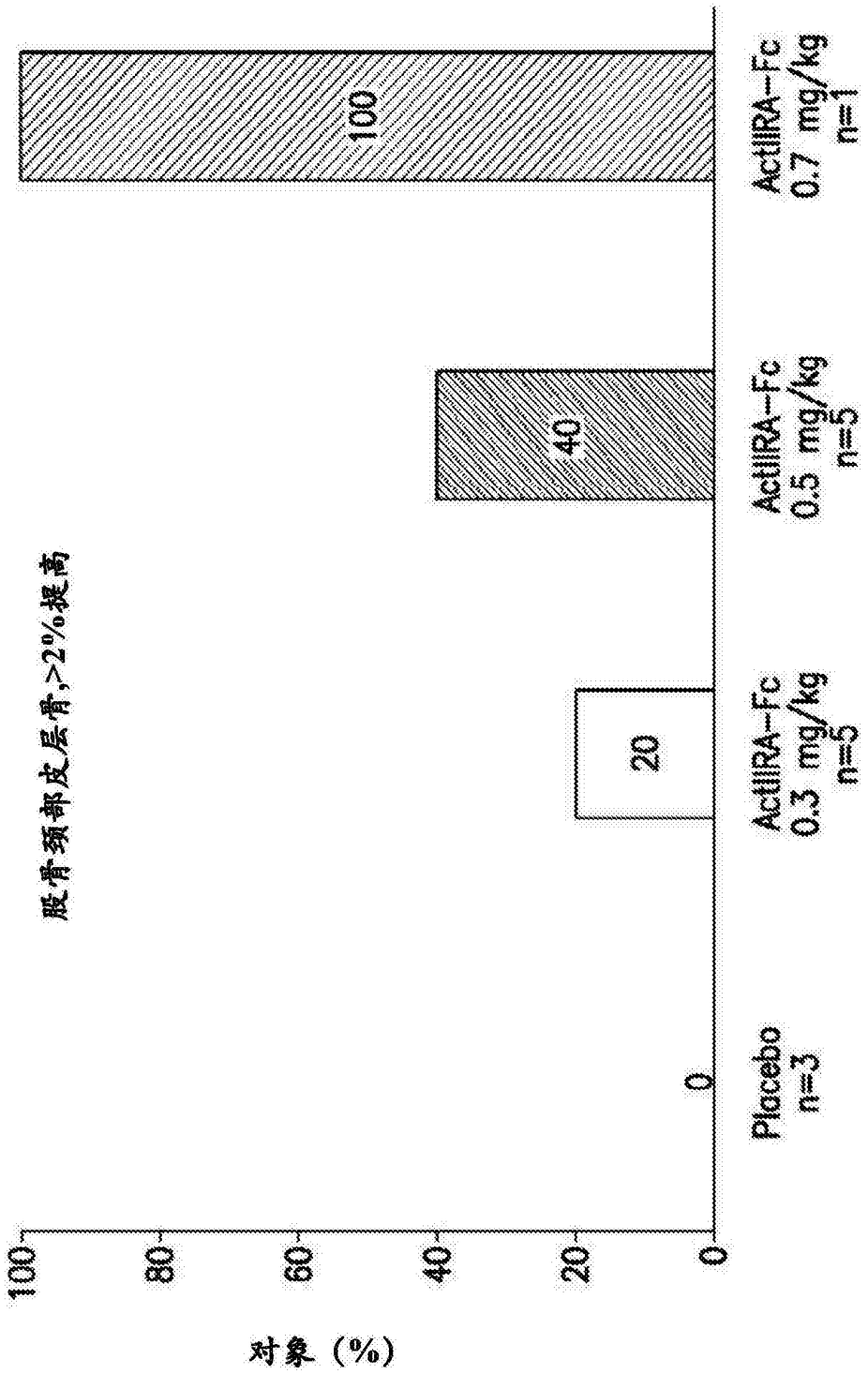

股骨颈部皮层骨,>2%提高
对象（%）
100
80
60
40
20
0
100
40
20
0
Placebo n=3
ActIIRA-Fc 0.3 mg/kg n=5
ActIIRA-Fc 0.5 mg/kg n=5
ActIIRA-Fc 0.7 mg/kg n=1

图9A

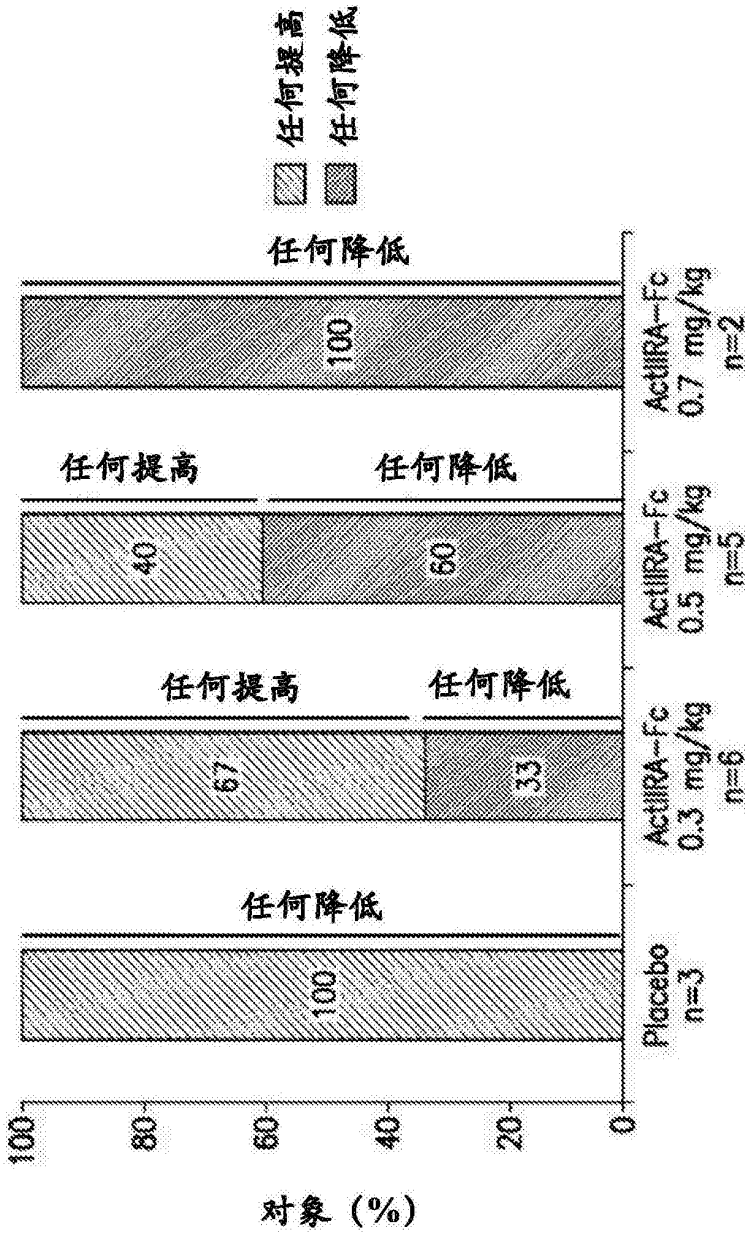

任何提高
任何降低
任何降低
100
ActIIRA-Fc
0.7 mg/kg
n=2
任何提高
任何降低
40
60
ActIIRA-Fc
0.5 mg/kg
n=5
任何提高
任何降低
67
33
ActIIRA-Fc
0.3 mg/kg
n=6
任何降低
100
Placebo
n=3
100
80
60
40
20
0
对象（%）

图9B

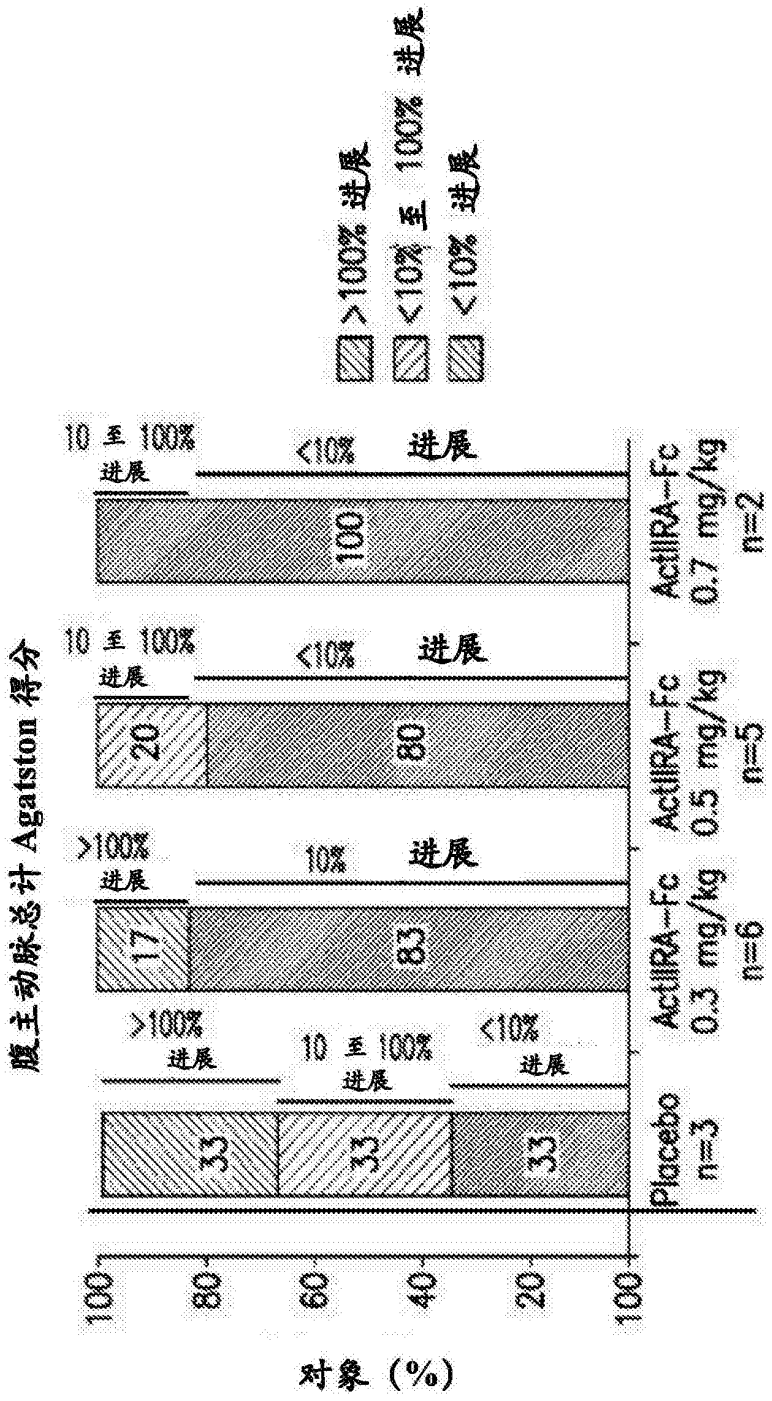

>100% 进展
<10% 至 100% 进展
<10% 进展
腹主动脉总计 Agatston 得分
10 至 100% 进展
<10% 进展
100
ActIIRA-Fc 0.7 mg/kg n=2
10 至 100% 进展
<10% 进展
20
80
ActIIRA-Fc 0.5 mg/kg n=5
>100% 进展
10% 进展
17
83
ActIIRA-Fc 0.3 mg/kg n=6
>100% 进展
10 至 100% 进展
<10% 进展
33
33
33
Placebo n=3
100
80
60
40
20
100
对象 (%)

图10A

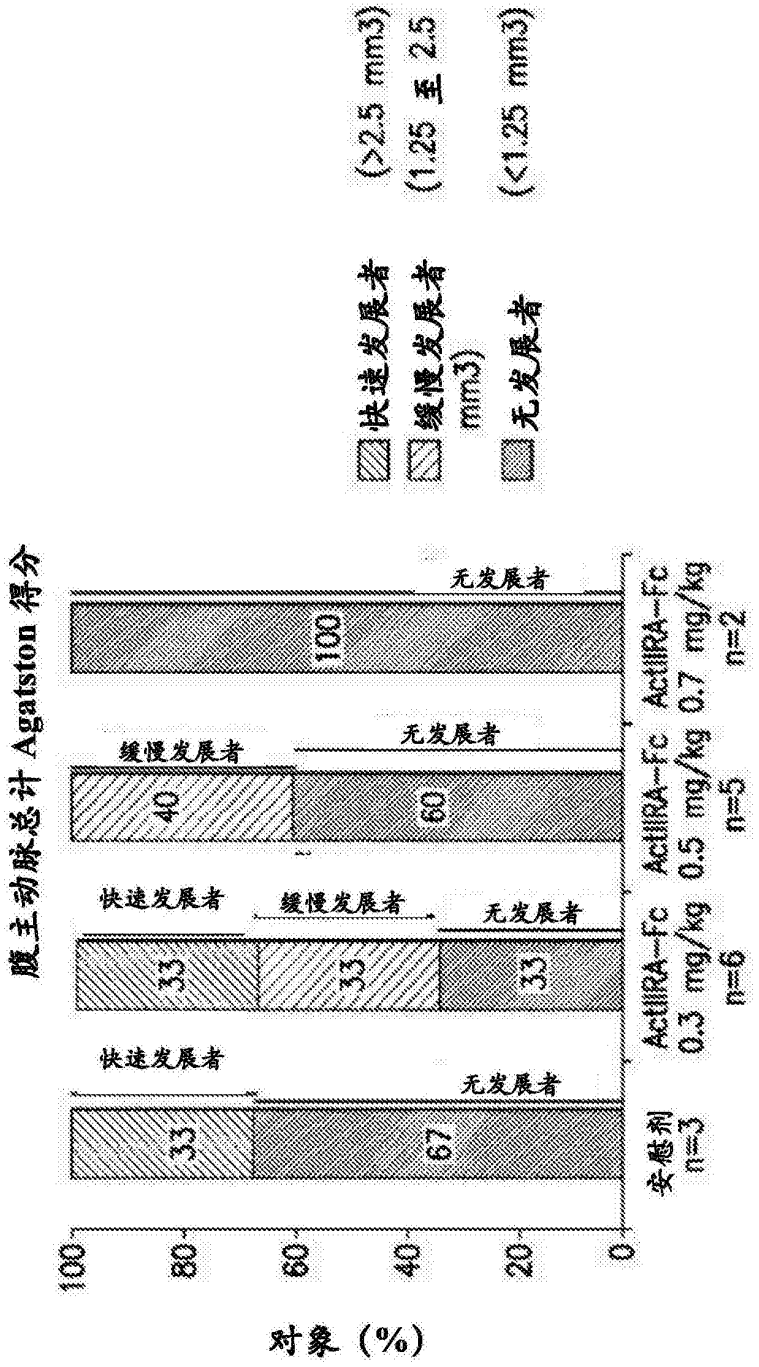

(>2.5 mm3)
(1.25 至 2.5
(<1.25 mm3)
快速发展者
缓慢发展者
mm3)
无发展者
腹主动脉总计 Agatston 得分
无发展者
100
缓慢发展者
无发展者
40
60
快速发展者
缓慢发展者
无发展者
33
33
33
快速发展者
无发展者
33
67
ActIIRA-Fc 0.7 mg/kg n=2
ActIIRA-Fc 0.5 mg/kg n=5
ActIIRA-Fc 0.3 mg/kg n=6
安慰剂 n=3
100
80
60
40
20
0
对象（%）

图10B

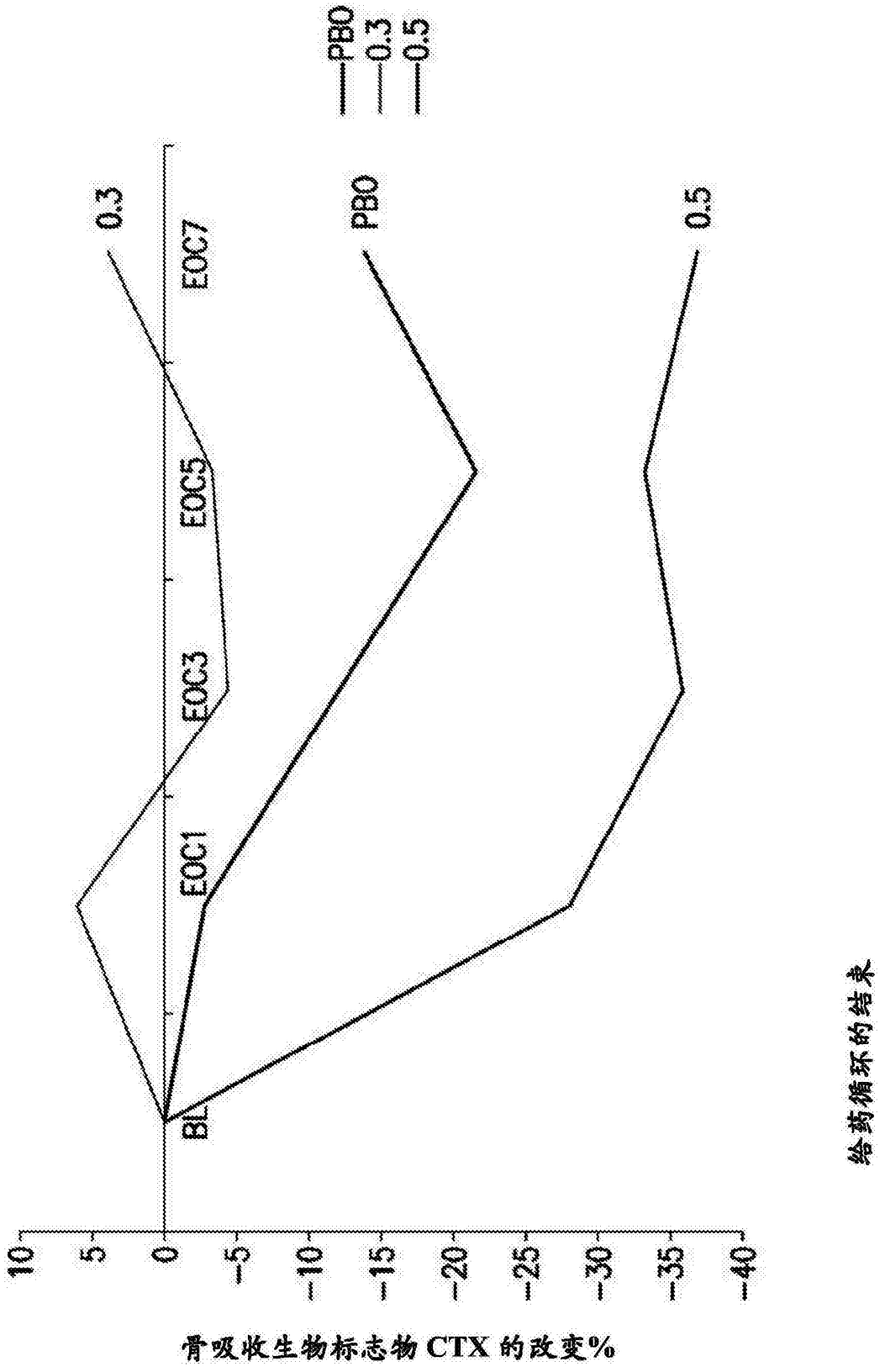

PBO
0.3
0.5
0.3
EOC7
EOC5
EOC3
EOC1
BL
PBO
0.5
10
5
0
−5
−10
−15
−20
−25
−30
−35
−40
骨吸收生物标志物 CTX 的改变%
给药循环的结束

图11

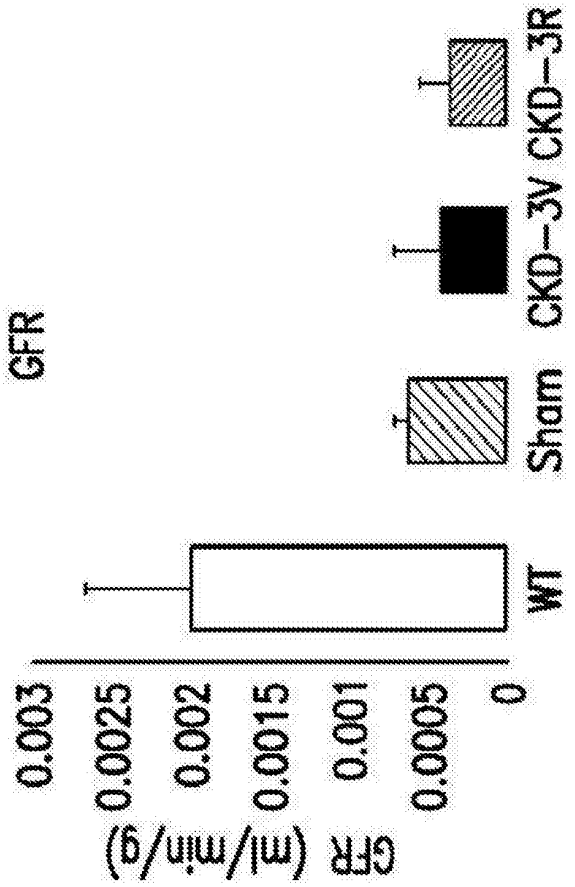

GFR
WT
Sham
CKD-3V
CKD-3R
0
0.0005
0.001
0.0015
0.002
0.0025
0.003
GFR (ml/min/g)

图12A

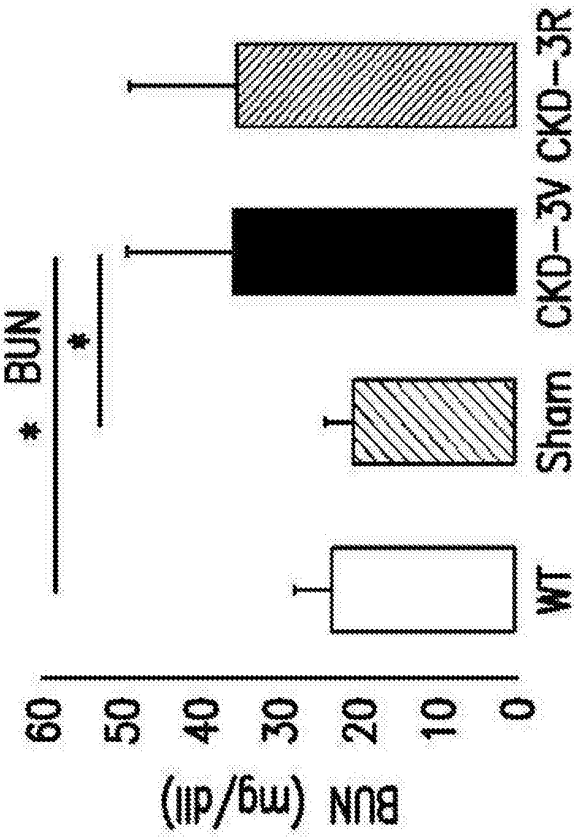


图12B

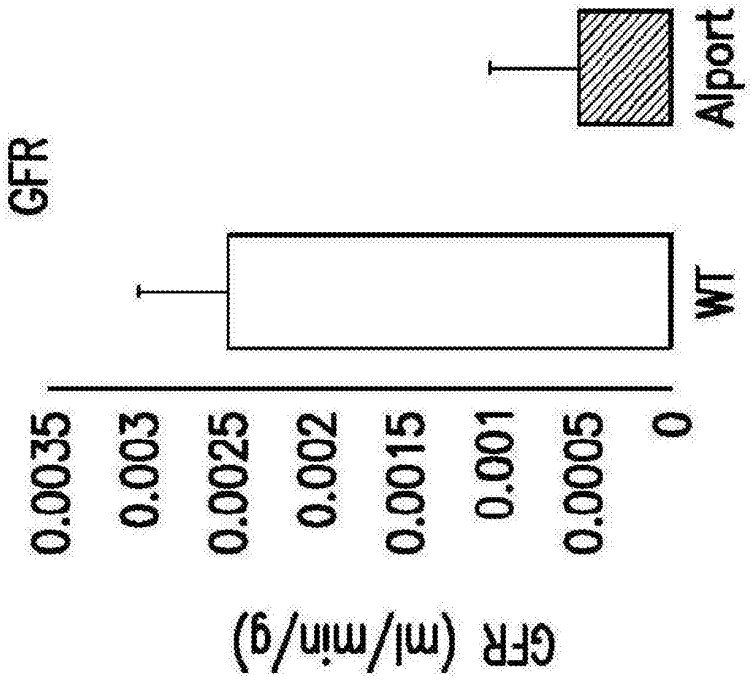


图12C

320

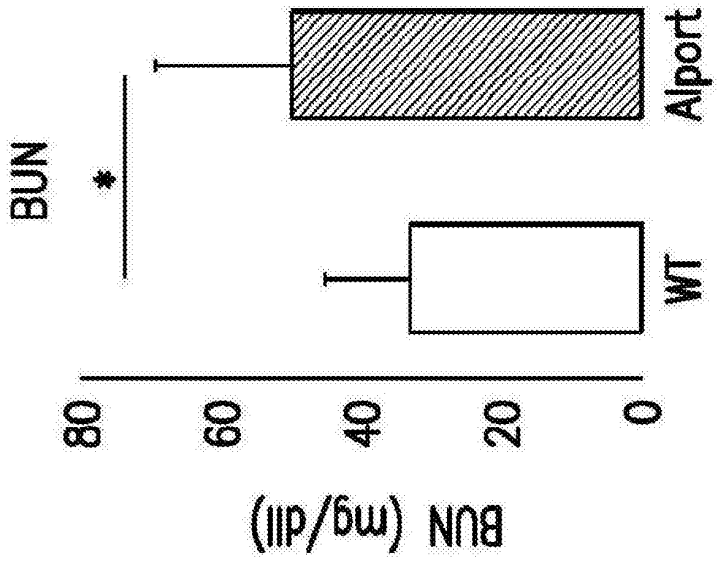


图12D

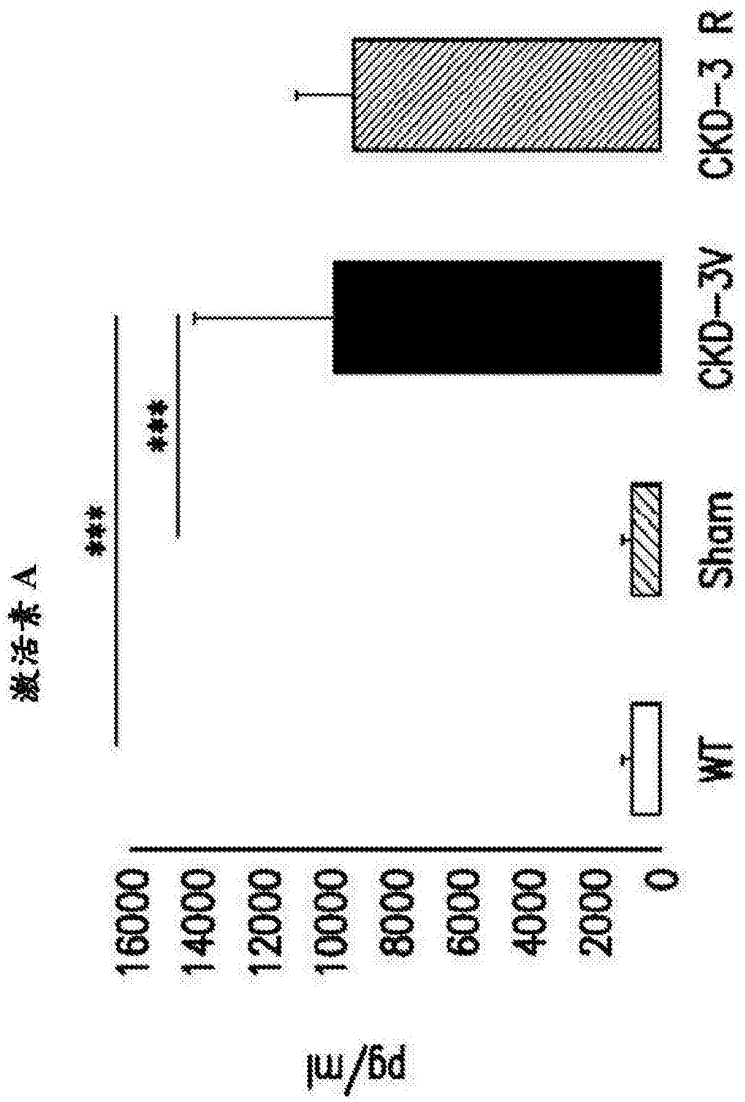

CKD-3 R
CKD-3V
Sham
WT
***
***
激活素 A
16000
14000
12000
10000
8000
6000
4000
2000
0
pg/ml

图13A

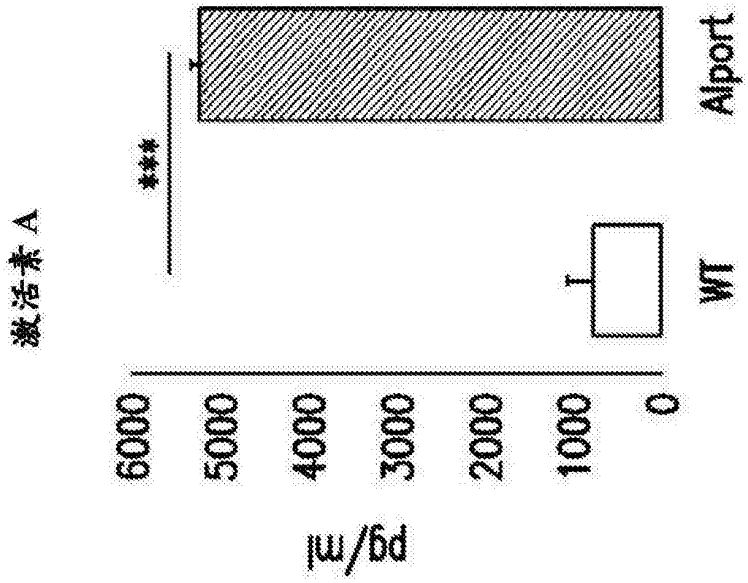


图13B

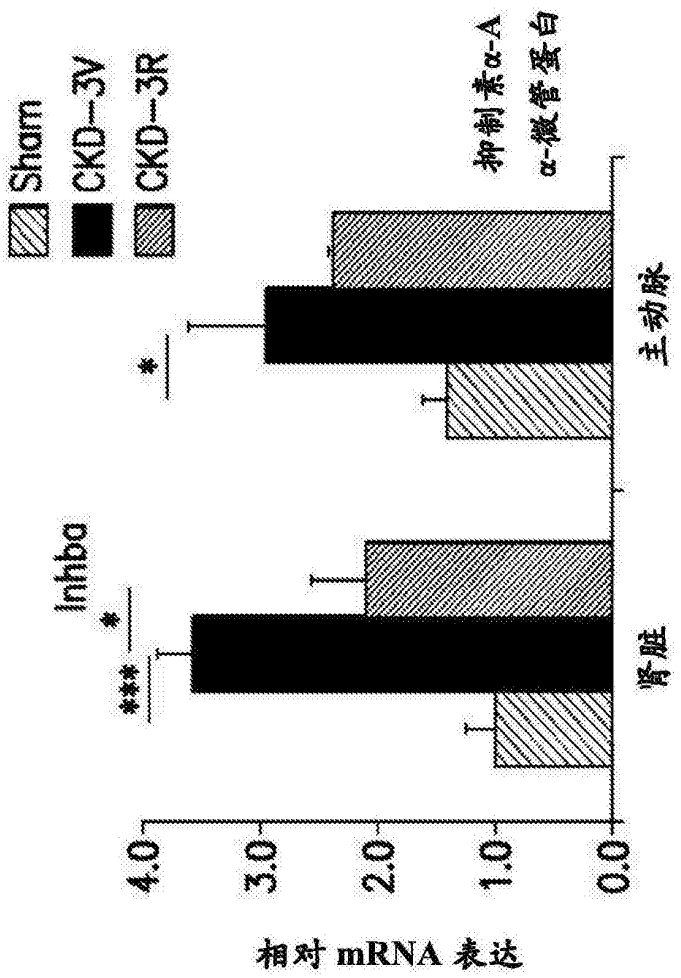

Sham
CKD-3V
CKD-3R
抑制素α-A
α-微管蛋白
主动脉
肾脏
Inhba
*
***
4.0
3.0
2.0
1.0
0.0
相对 mRNA 表达

图13C

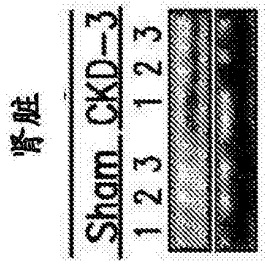

肾脏
Sham CKD-3
1 2 3 1 2 3

图13D

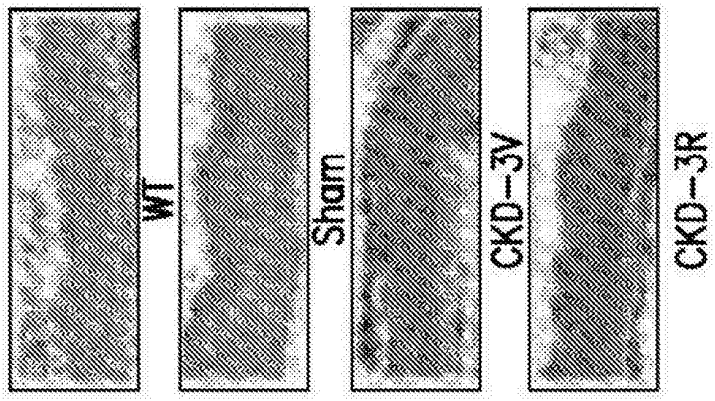

WT
Sham
CKD-3V
CKD-3R

图13E

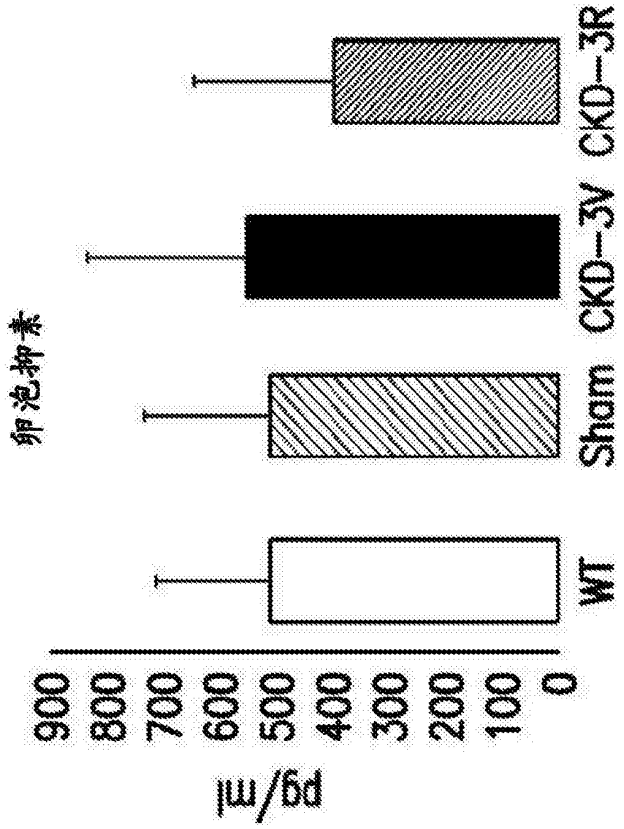

卵泡抑素
WT
Sham
CKD-3V
CKD-3R
0
100
200
300
400
500
600
700
800
900
pg/ml

图13F

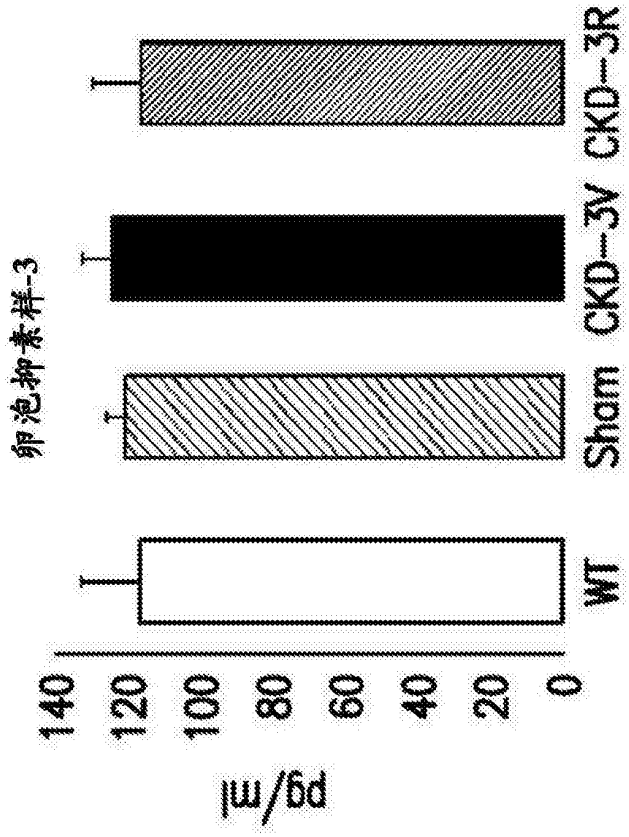


图13G

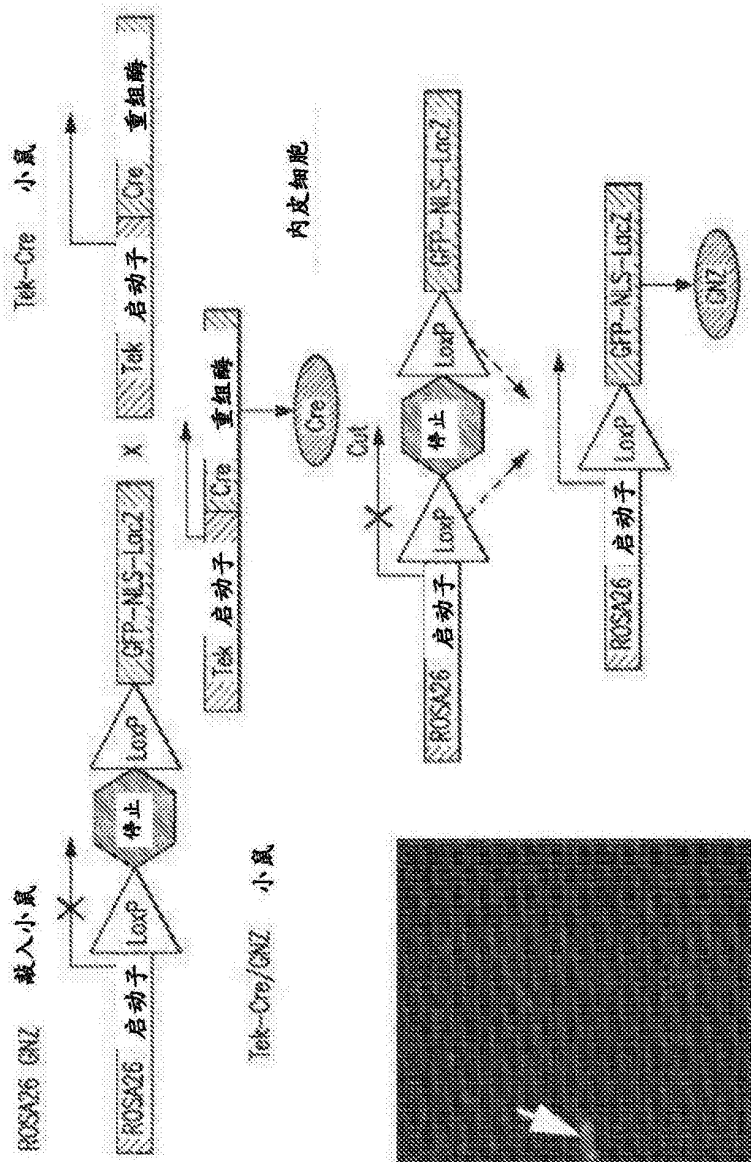

ROSA26 GNZ 载入小鼠
Tek-Cre 小鼠
Tek-Cre/GNZ 小鼠
内皮细胞
ROSA26
启动子
LoxP
终止
GFP-NLS-LacZ
Tek
Cre
重组酶
Cre
Cut
GNZ

图 14A

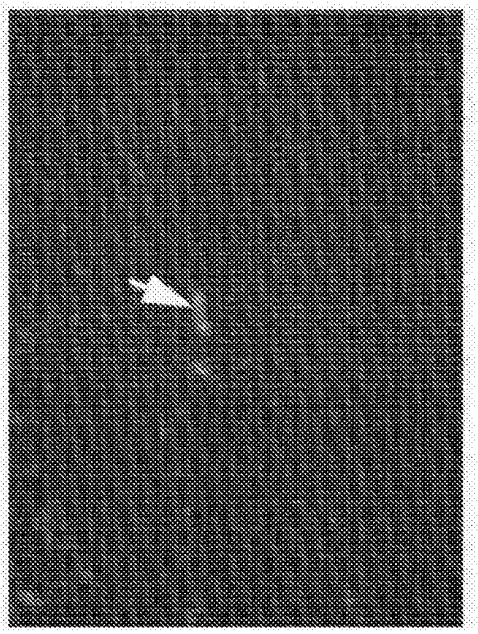


图 14B

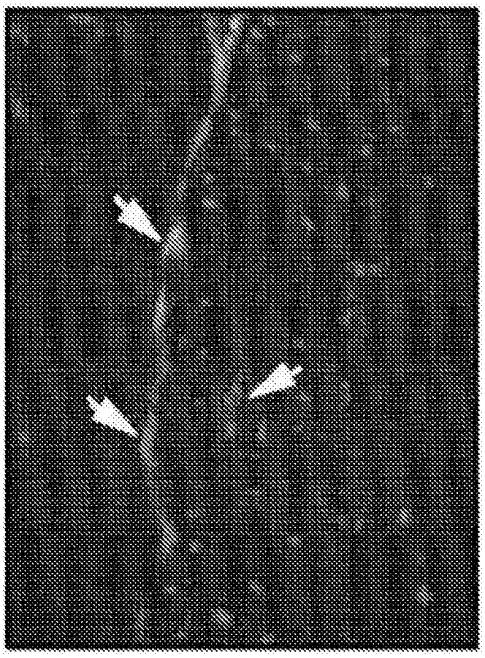


图14C

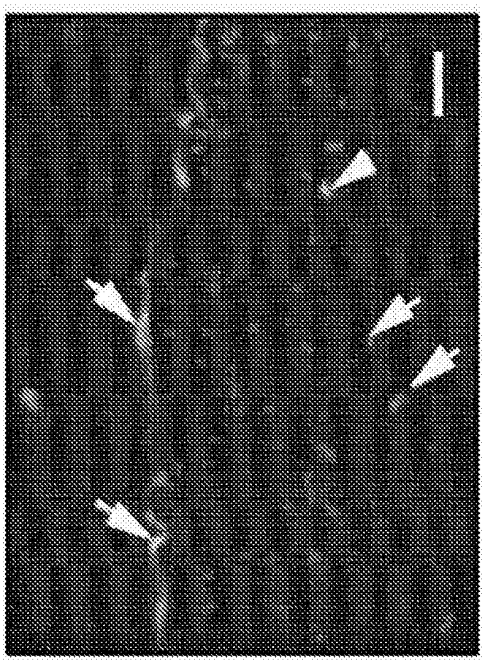


图14D

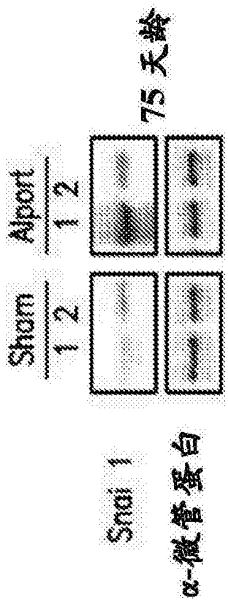


图14E

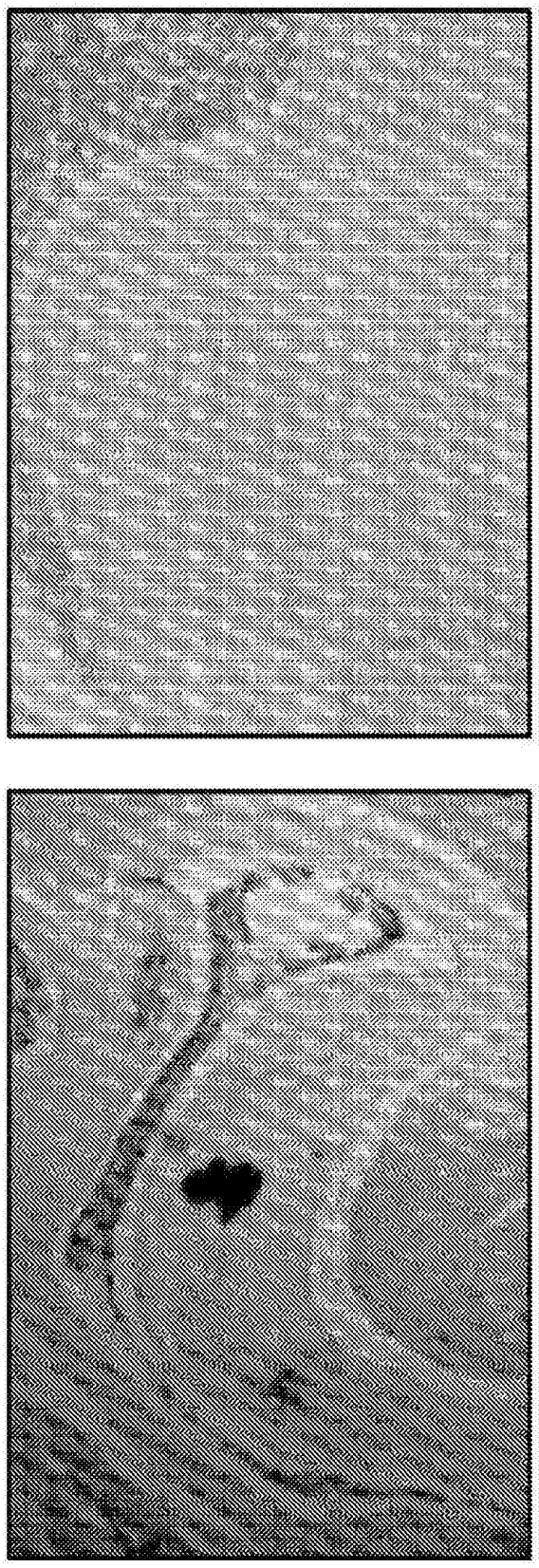


图15A

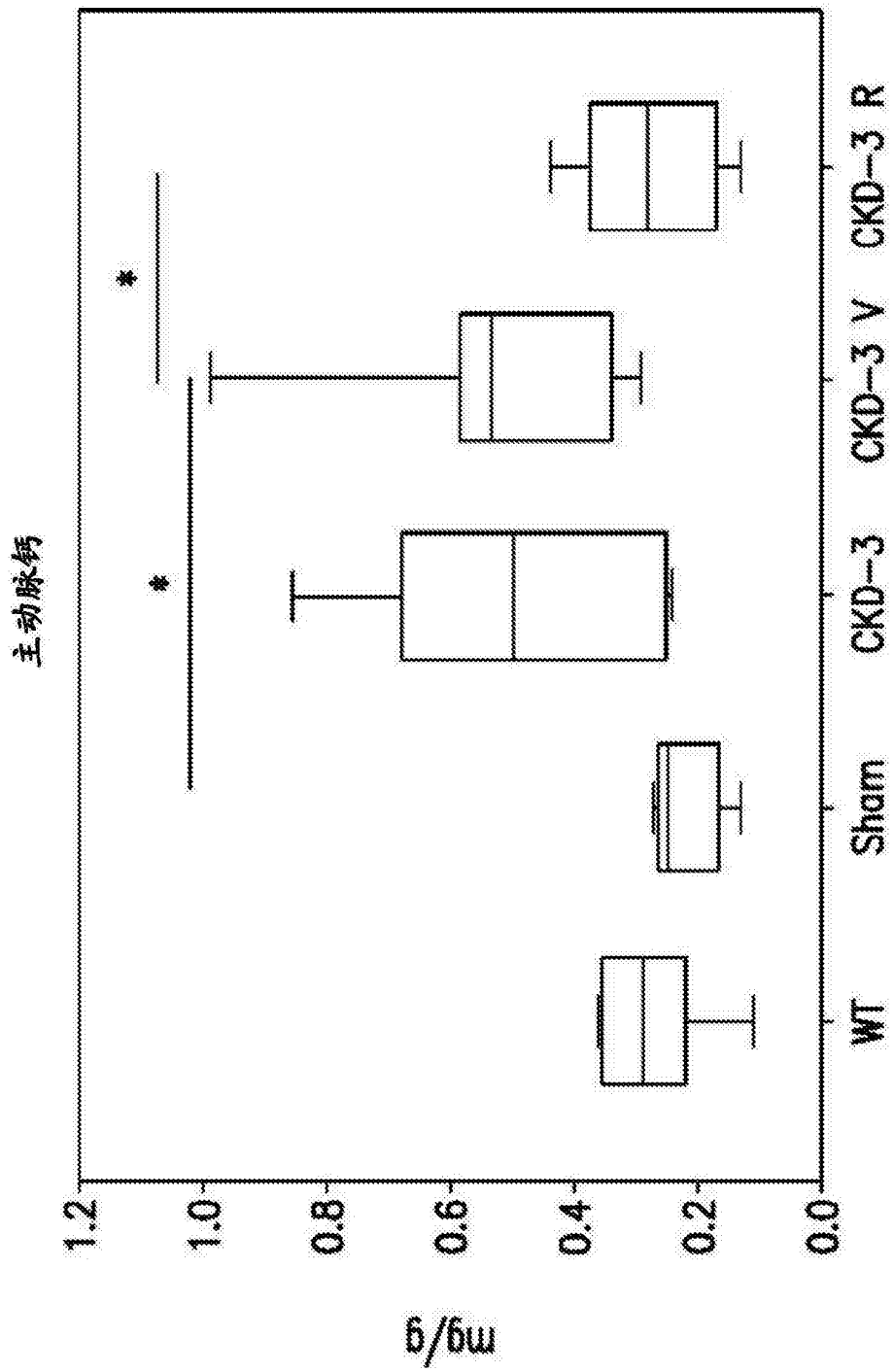


图15B

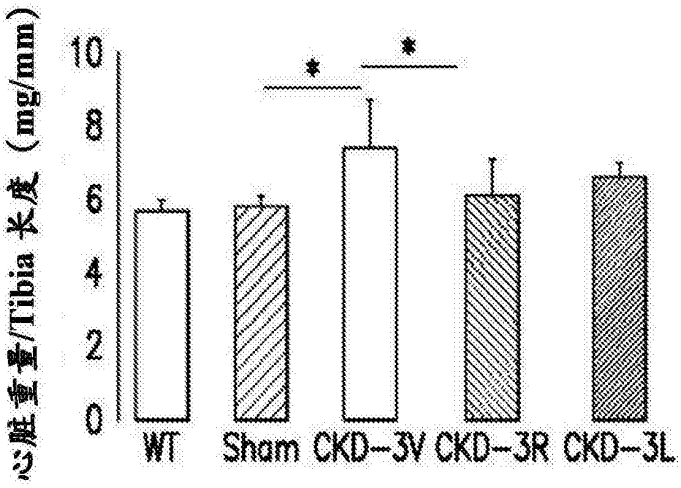


图16A

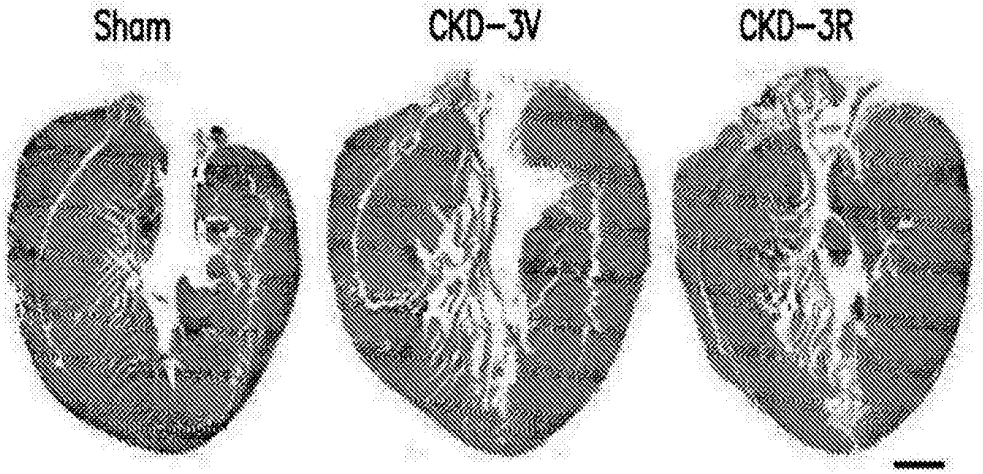


图16B

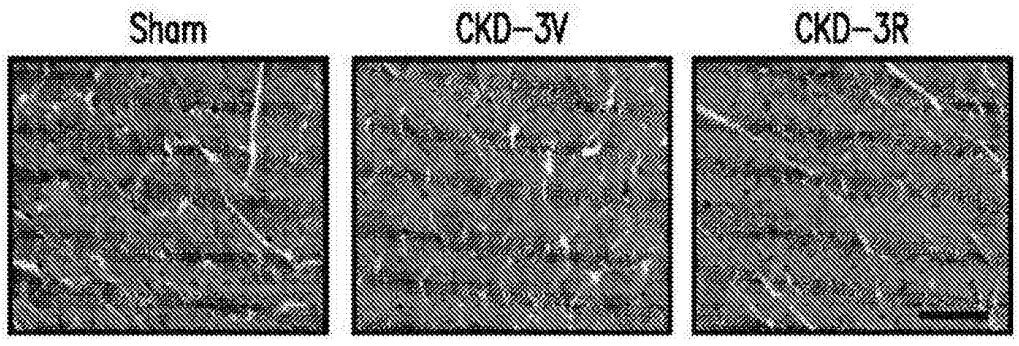


图16C

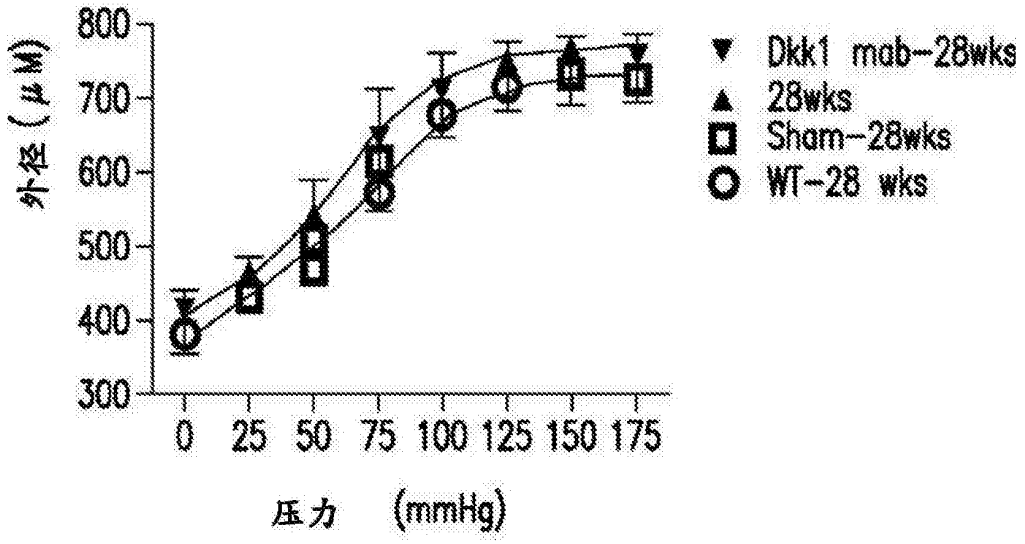

外径（μM）
800
700
600
500
400
300
0 25 50 75 100 125 150 175
压力 (mmHg)
Dkk1 mab-28wks
28wks
Sham-28wks
WT-28 wks

图17A

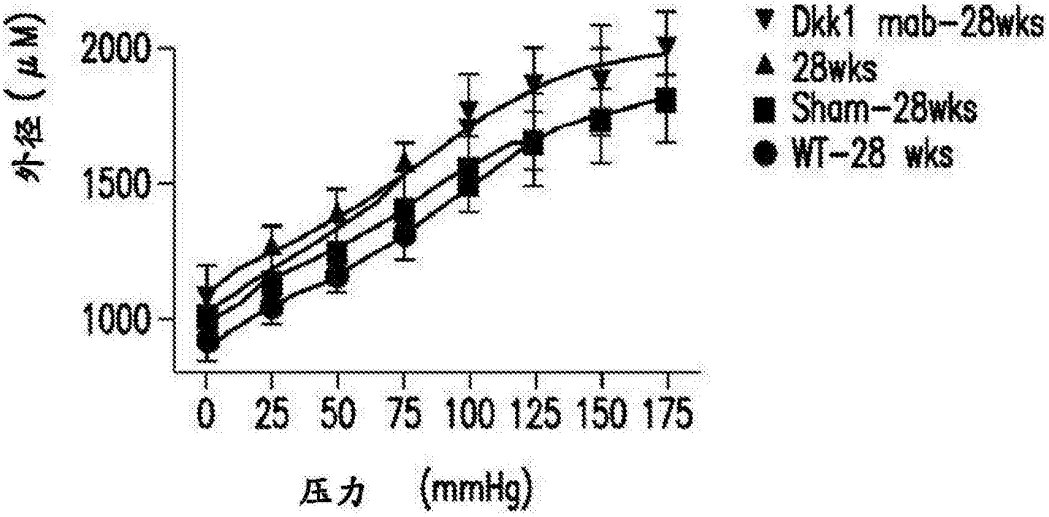

外径（μM）
2000
1500
1000
0 25 50 75 100 125 150 175
压力 (mmHg)
Dkk1 mab-28wks
28wks
Sham-28wks
WT-28 wks

图17B

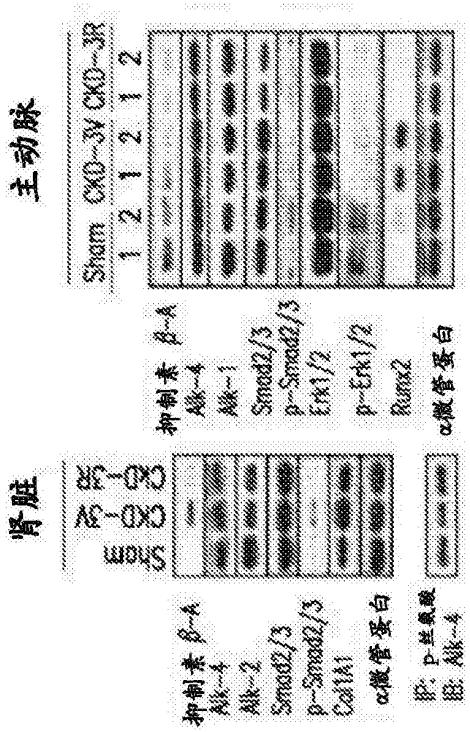

主动脉
Sham CKO-3V CKO-3R
1 2 1 2 1 2
抑制素 β-A
Alk-4
Alk-1
Smad2/3
p-Smad2/3
Erk1/2
p-Erk1/2
Runx2
α微管蛋白
肾脏
Sham
CKO-3V
CKO-3R
抑制素 β-A
Alk-4
Alk-2
Smad2/3
p-Smad2/3
Col1A1
α微管蛋白
IP: p-丝氨酸
IB: Alk-4

图18A

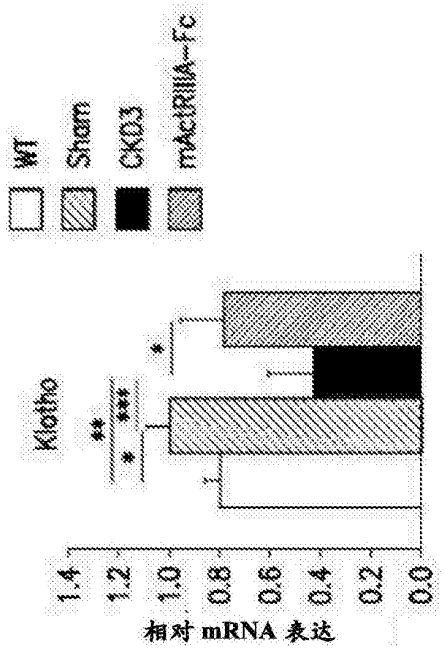
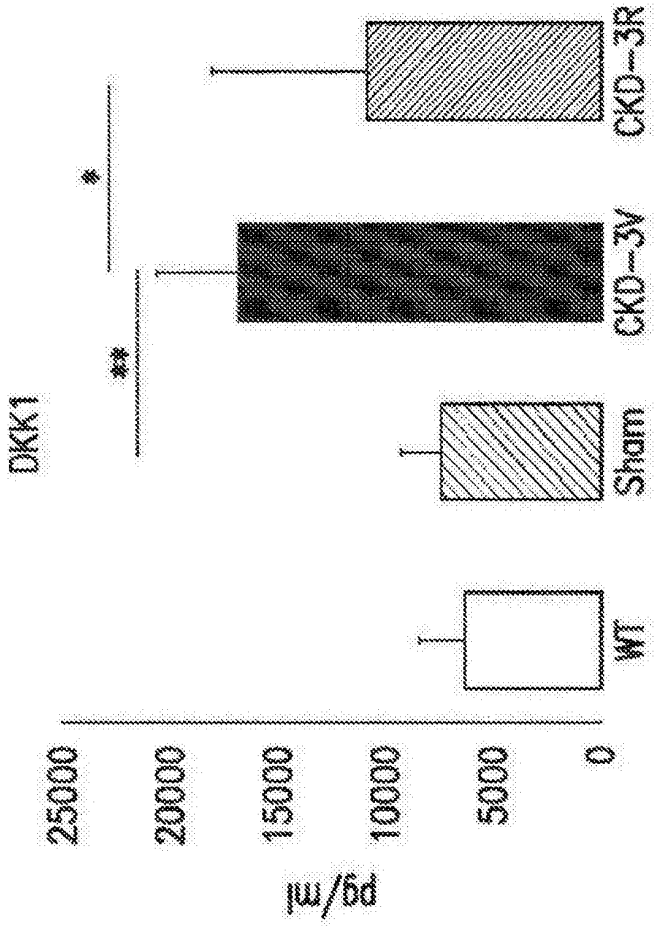

WT
Sham
CKO3
mActRIIIA-Fc
Klotho
**
***
*
*
1
1
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
相对 mRNA 表达

图18B


DKK1
pg/ml
25000
20000
15000
10000
5000
0
WT
Sham
CKD-3V
CKD-3R
**
*

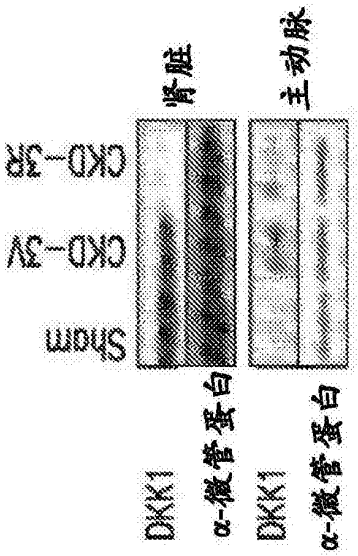

肾脏
主动脉
Sham
CKD-3V
CKD-3R
DKK1
α-微管蛋白
DKK1
α-微管蛋白

图18C

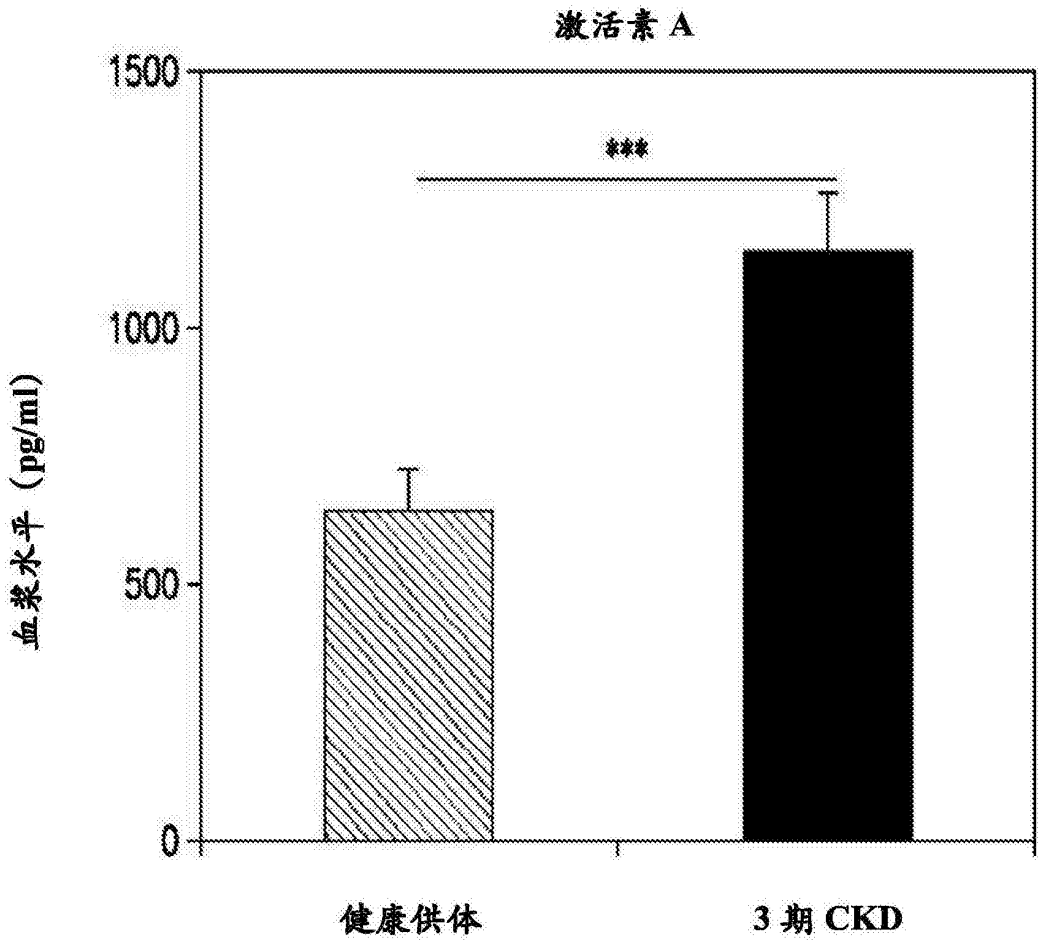


图19

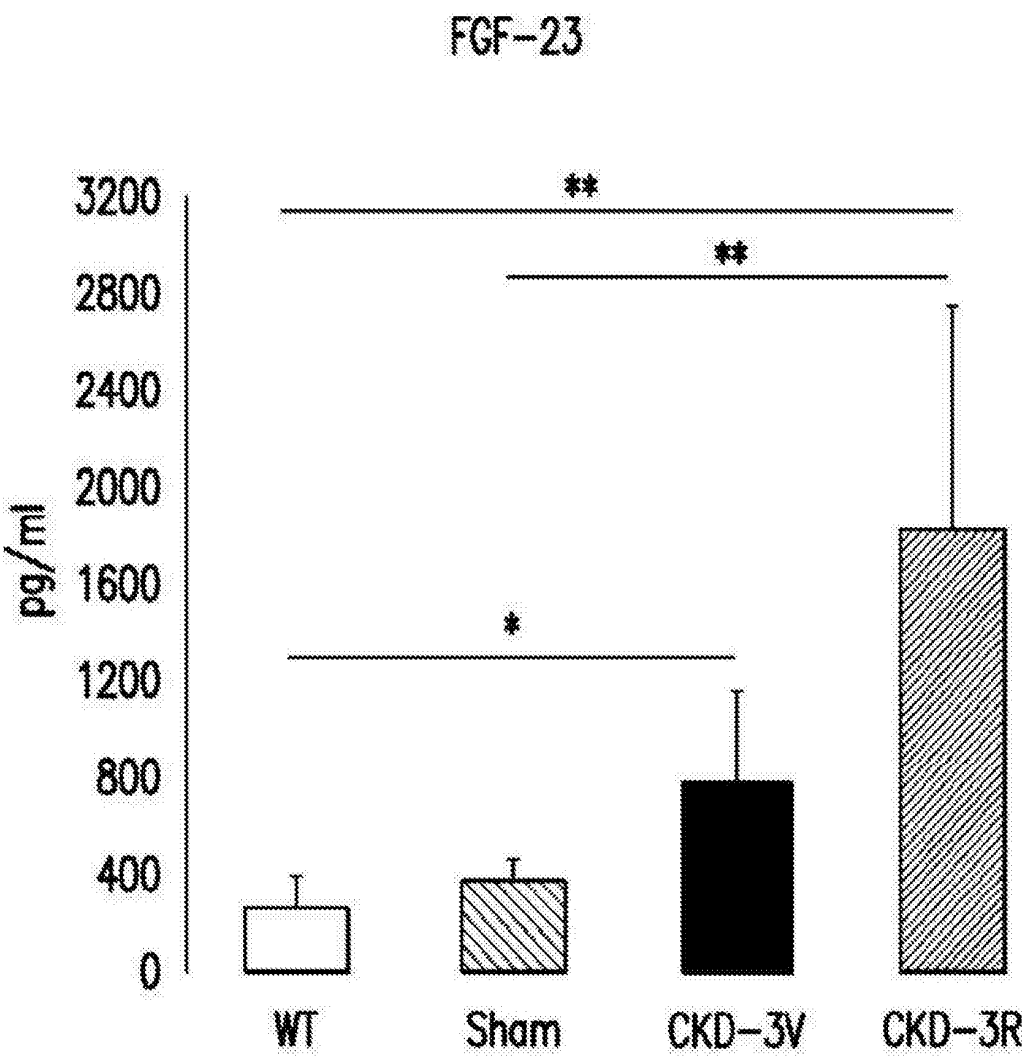

FGF-23
3200
2800
2400
2000
1600
1200
800
400
0
pg/ml
**
**
*
WT
Sham
CKD-3V
CKD-3R

图20

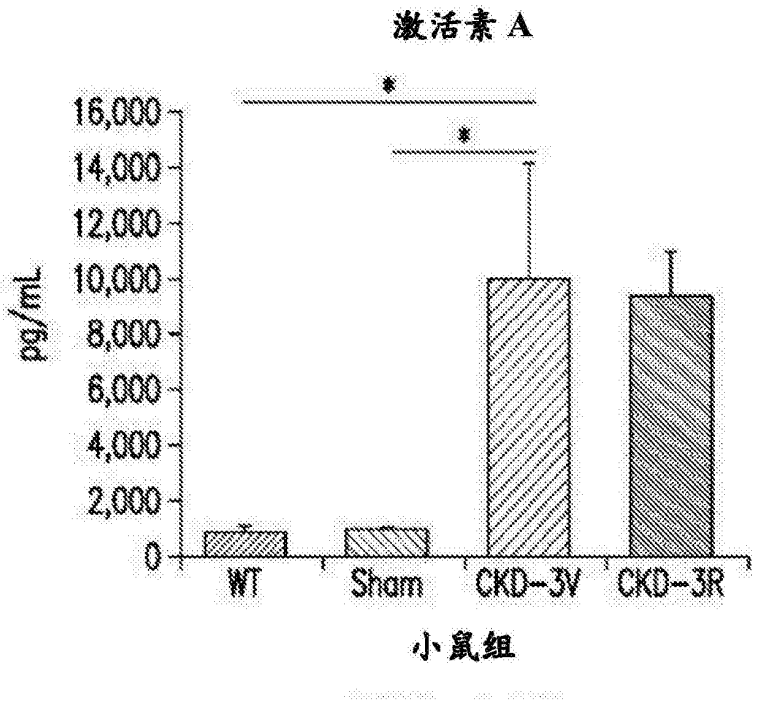

激活素 A
*
*
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
pg/mL
WT
Sham
CKD-3V
CKD-3R
小鼠组

图21A

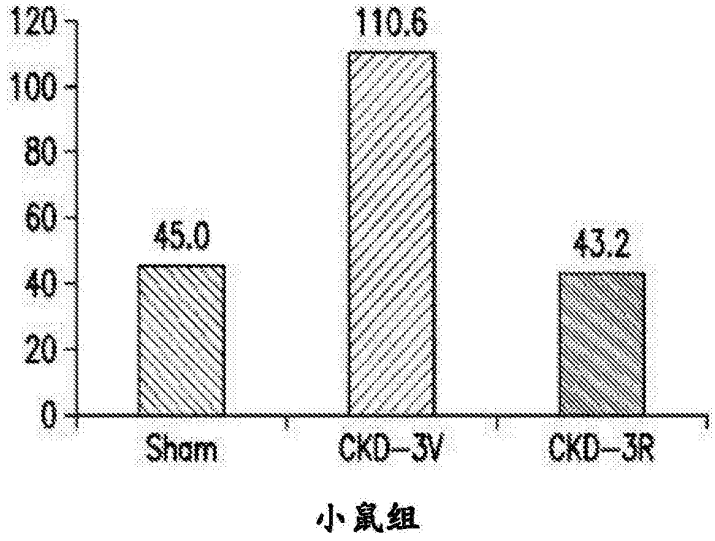

120
100
80
60
40
20
0
45.0
110.6
43.2
Sham
CKD-3V
CKD-3R
小鼠组

图21B

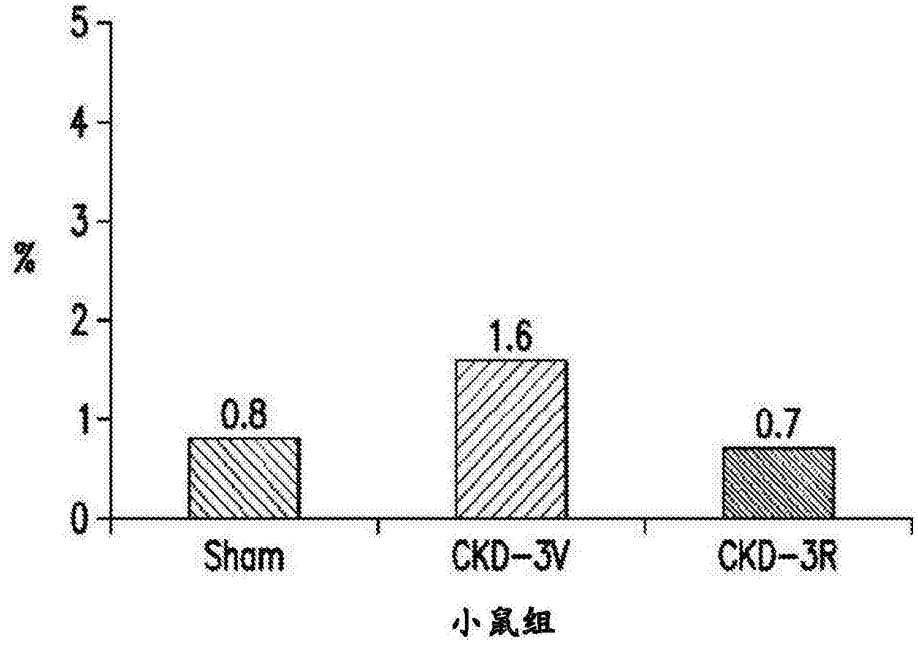

5
4
3
2
1
0
%
0.8
1.6
0.7
Sham
CKD-3V
CKD-3R
小鼠组

图21C

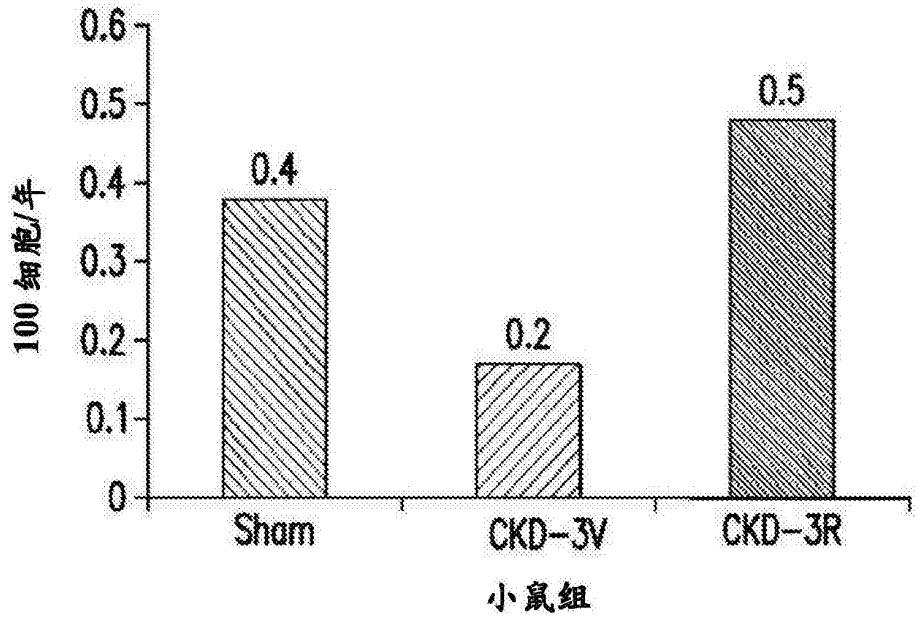
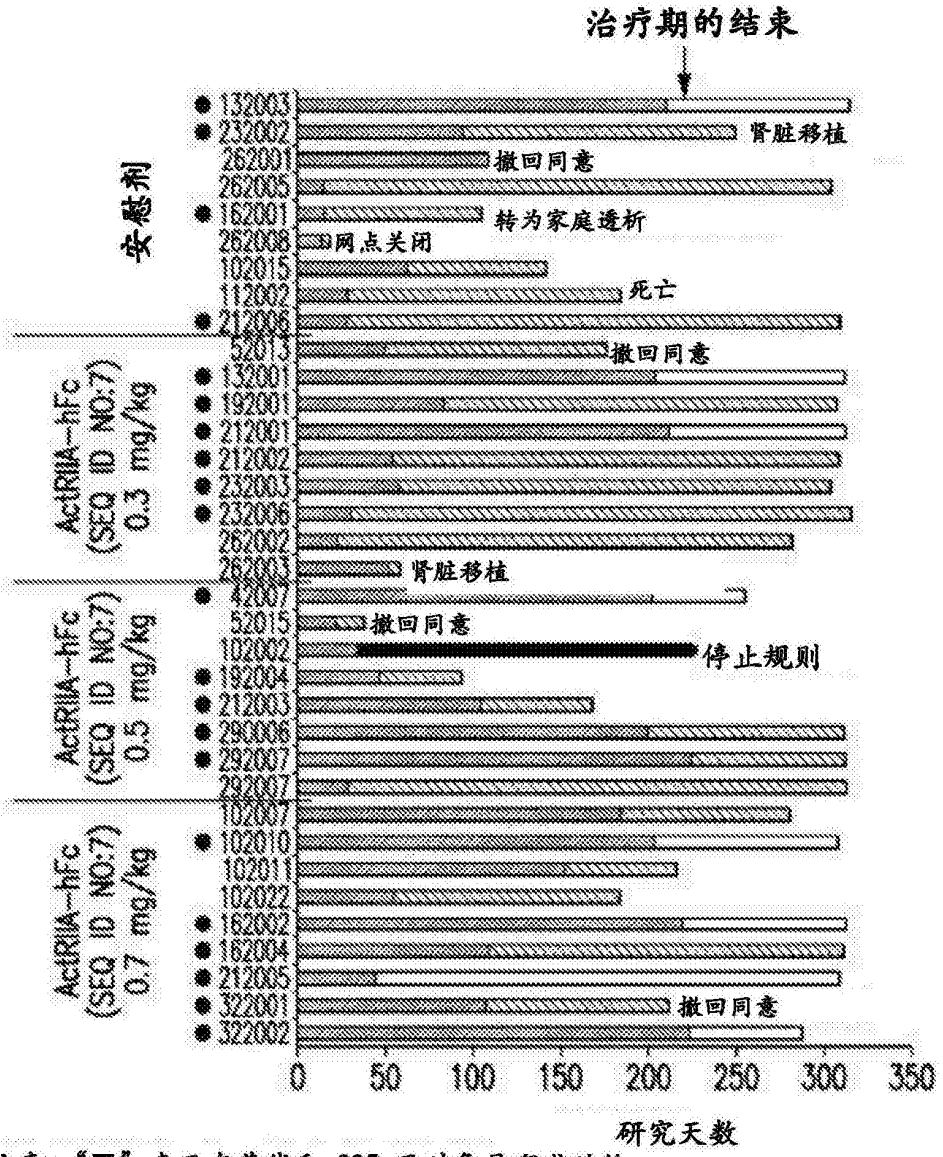

0.6
0.5
0.4
0.3
0.2
0.1
0
100 细胞/年
0.4
0.2
0.5
Sham
CKD-3V
CKD-3R
小鼠组

图21D



注意:“▼”表示在基线和 225 天对象具有成对的 QCT。

*一名安慰剂对象在研究的 108 天撤回同意，在 148 天死亡；接受挽救治疗的 1 名安慰剂对象在 186 天死亡。

违反协议

受试者在 29 天满足升高的血液的停止标准；研究治疗停止，在 36 天施用挽救治疗，继续跟踪。

基于基线时不完全的评估，对象随机化到有不合格血压错误中。

图22A

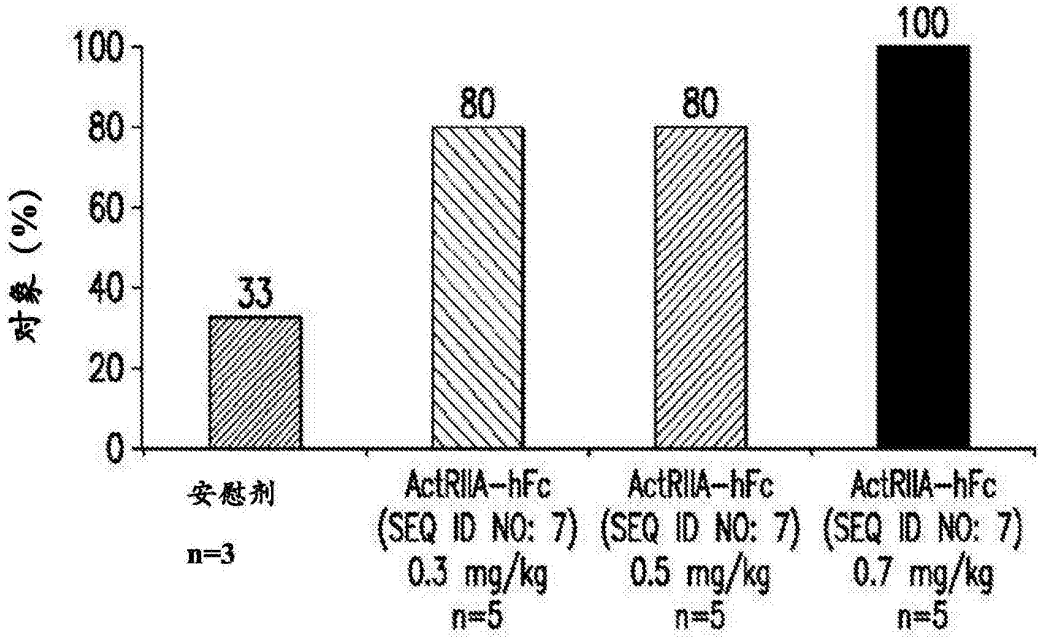

100
80
60
40
20
0
对象（%）
33
80
80
100
安慰剂
n=3
ActRIIA-hFc
(SEQ ID NO: 7)
0.3 mg/kg
n=5
ActRIIA-hFc
(SEQ ID NO: 7)
0.5 mg/kg
n=5
ActRIIA-hFc
(SEQ ID NO: 7)
0.7 mg/kg
n=5

图22B

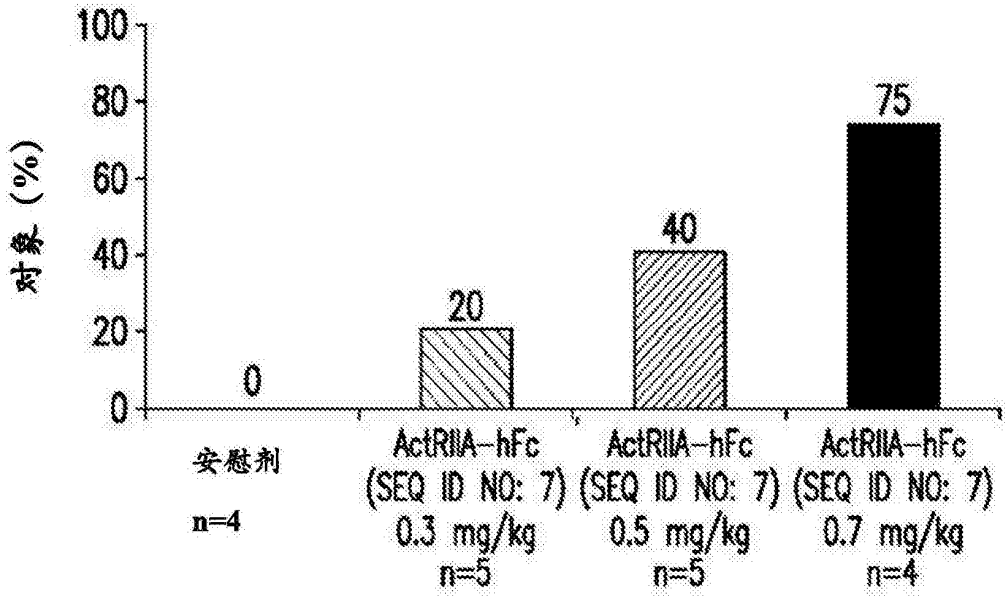

100
80
60
40
20
0
对象（%）
0
20
40
75
安慰剂
n=4
ActRIIA-hFc
(SEQ ID NO: 7)
0.3 mg/kg
n=5
ActRIIA-hFc
(SEQ ID NO: 7)
0.5 mg/kg
n=5
ActRIIA-hFc
(SEQ ID NO: 7)
0.7 mg/kg
n=4

图22C

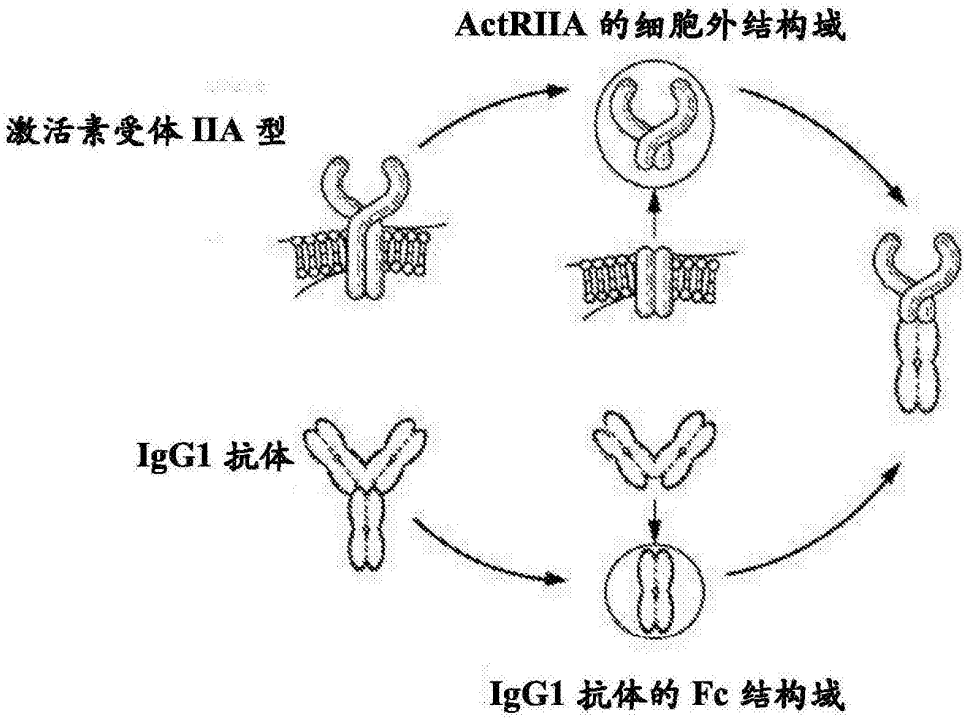

ActRIIA 的细胞外结构域
激活素受体 IIA 型
IgG1 抗体
IgG1 抗体的 Fc 结构域

图23A

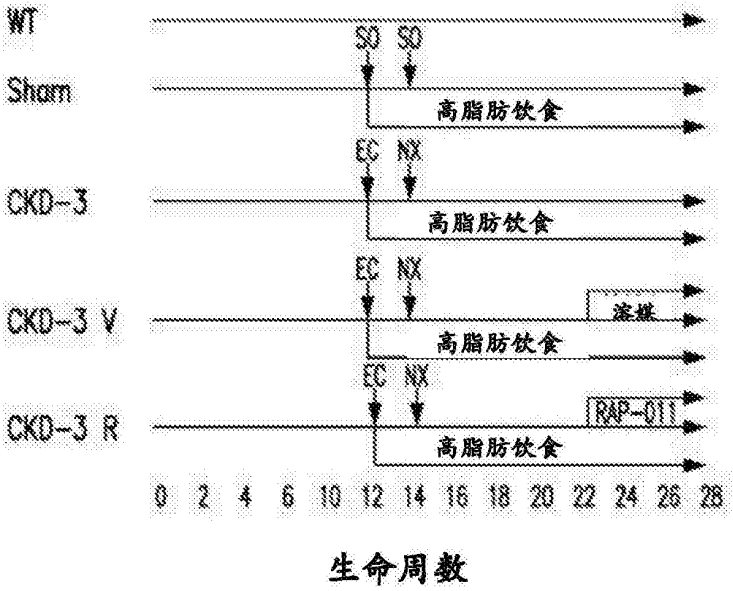

WT
SO SO
Sham
高脂肪饮食
EC NX
CKD-3
高脂肪饮食
EC NX
CKD-3 V
冻媒
高脂肪饮食
EC NX
CKD-3 R
RAP-011
高脂肪饮食
0 2 4 6 10 12 14 16 18 20 22 24 26 28
生命周数

图23B

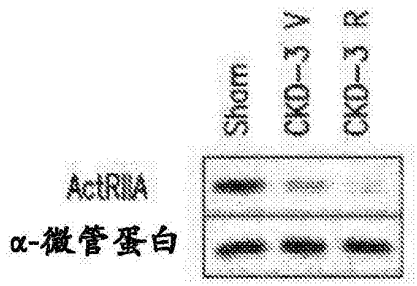


图24A

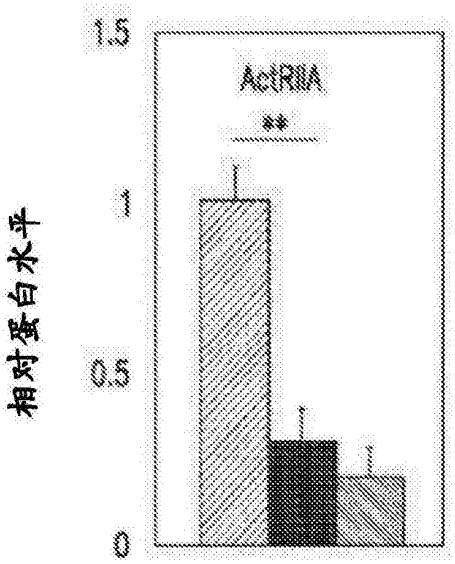


图24B

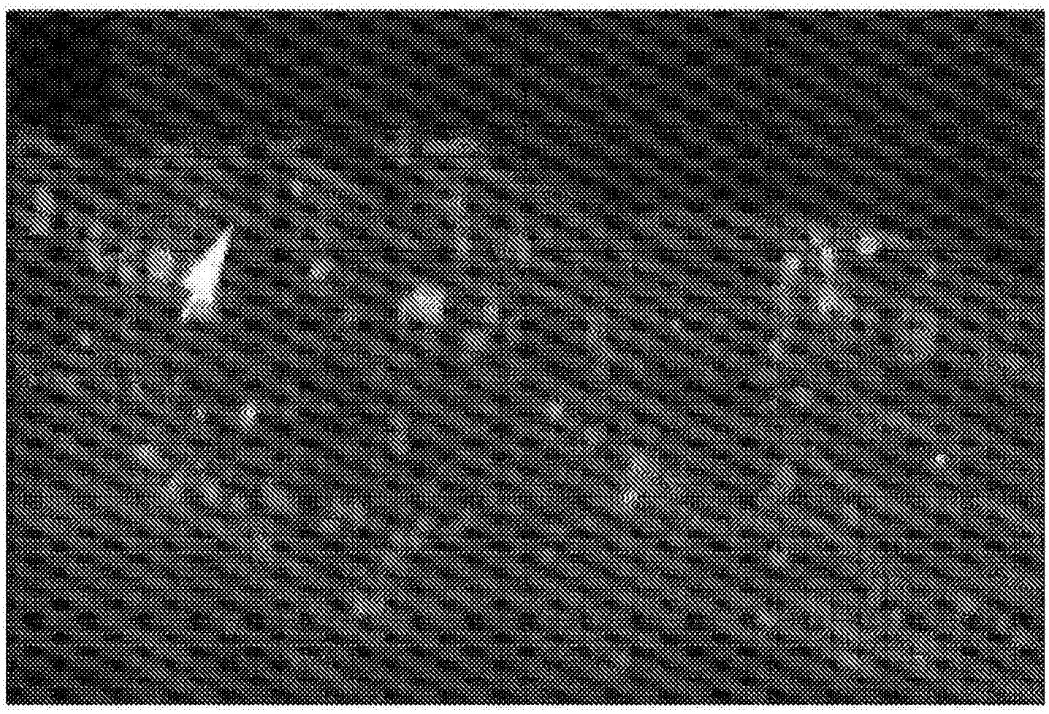


图24C

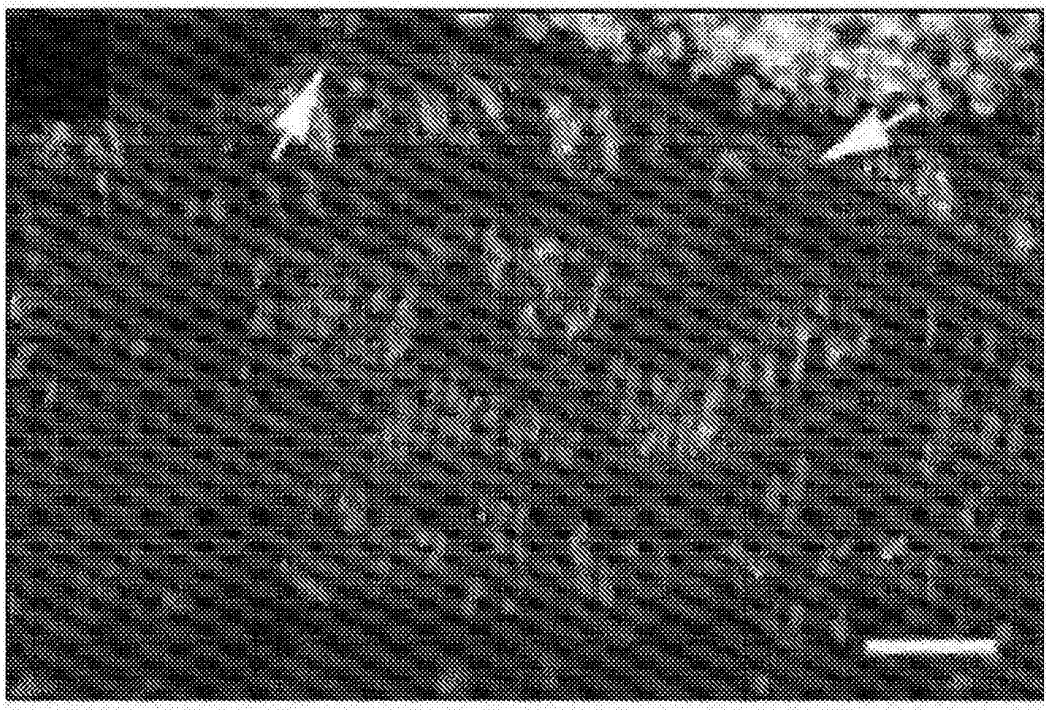


图24D

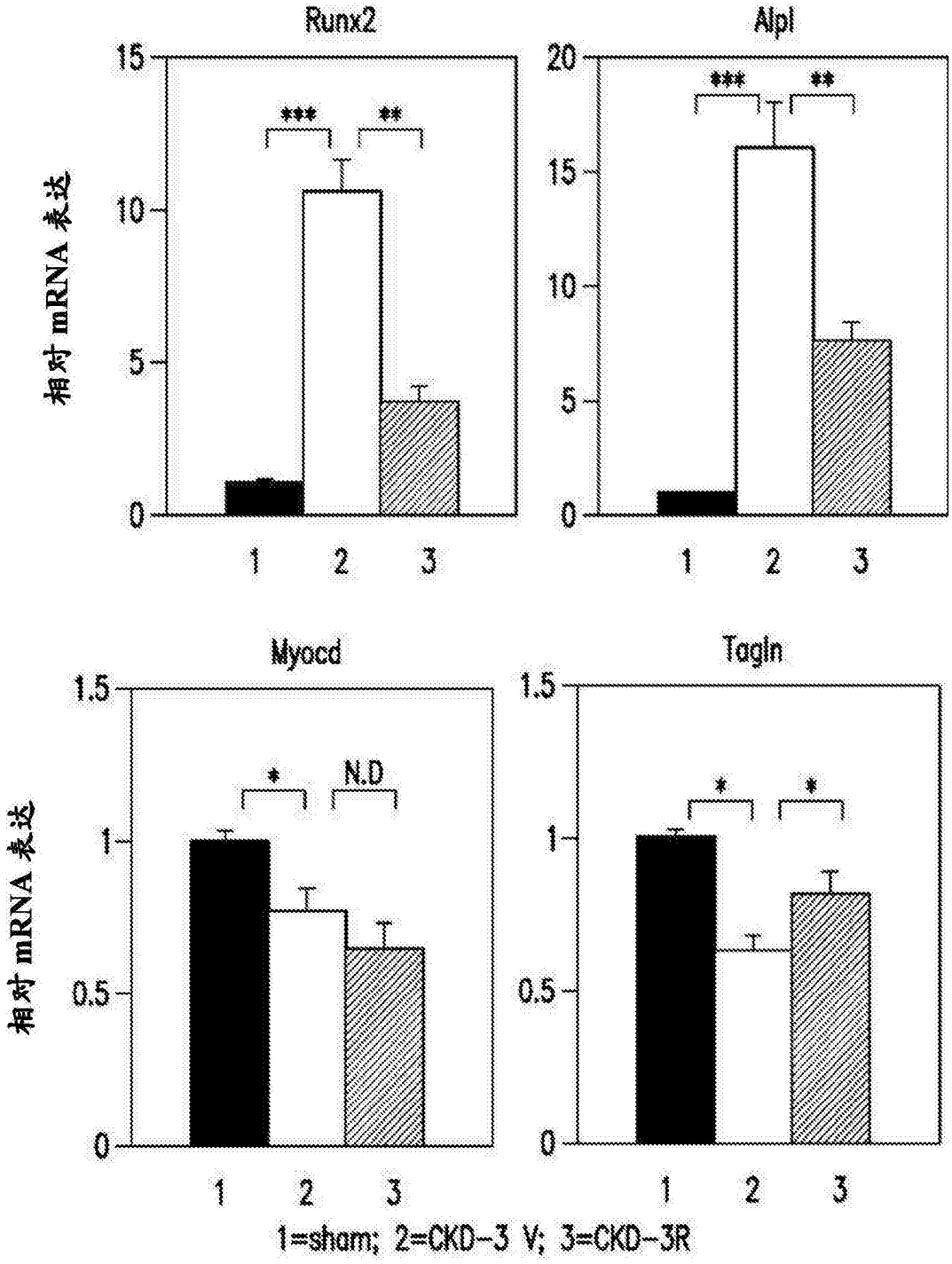

Runx2
Alpl
Myocd
Tagln
相对 mRNA 表达
相对 mRNA 表达
15
10
5
0
20
15
10
5
0
1.5
1
0.5
0
***
**
*
N.D
1 2 3
1=sham; 2=CKD-3 V; 3=CKD-3R

图25A

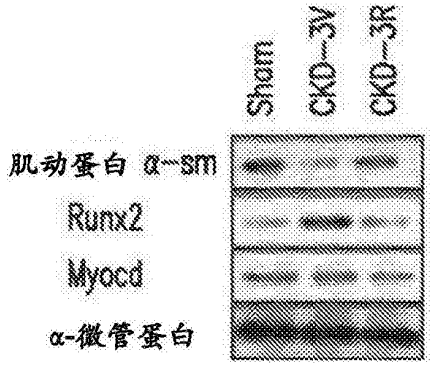

Sham
CKD-3V
CKD-3R
肌动蛋白 α-sm
Runx2
Myocd
α-微管蛋白

图25B

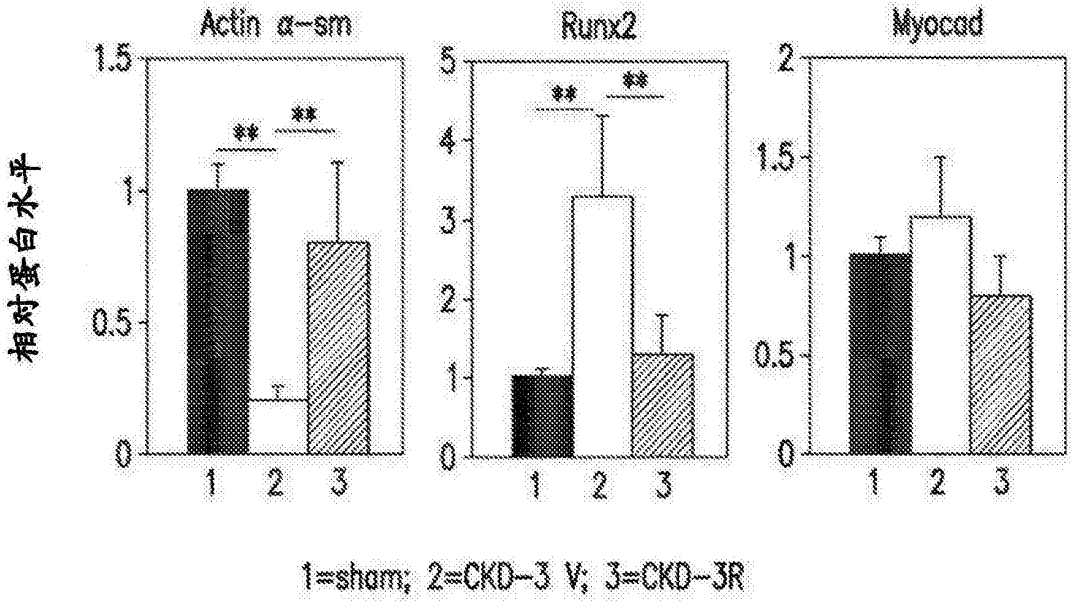

Actin α-sm
Runx2
Myocad
相对蛋白水平
1.5
1
0.5
0
5
4
3
2
1
0
2
1.5
1
0.5
0
**
**
**
**
1 2 3
1 2 3
1 2 3
1=sham; 2=CKD-3 V; 3=CKD-3R

图25C

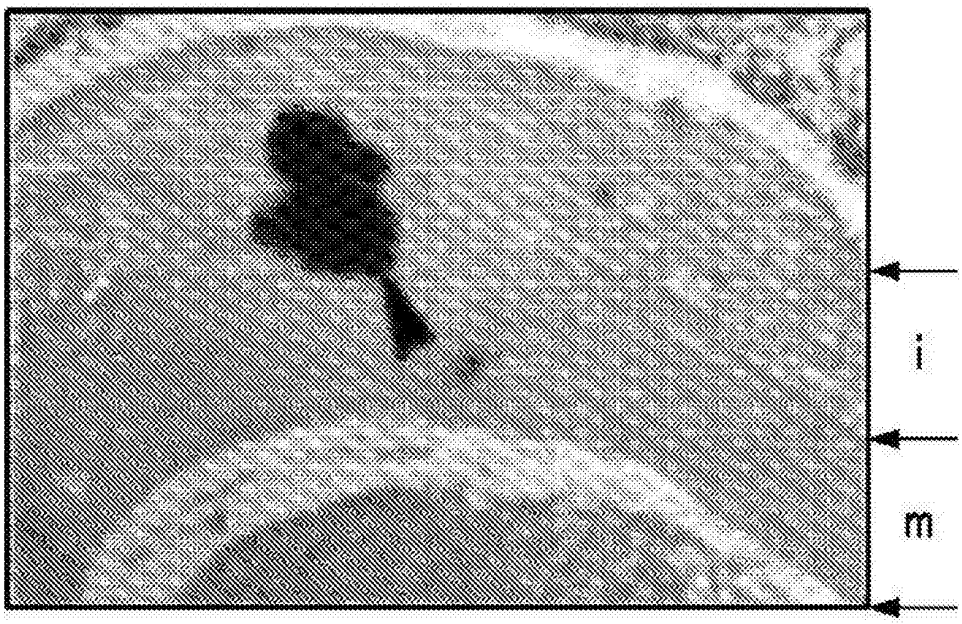


图26A

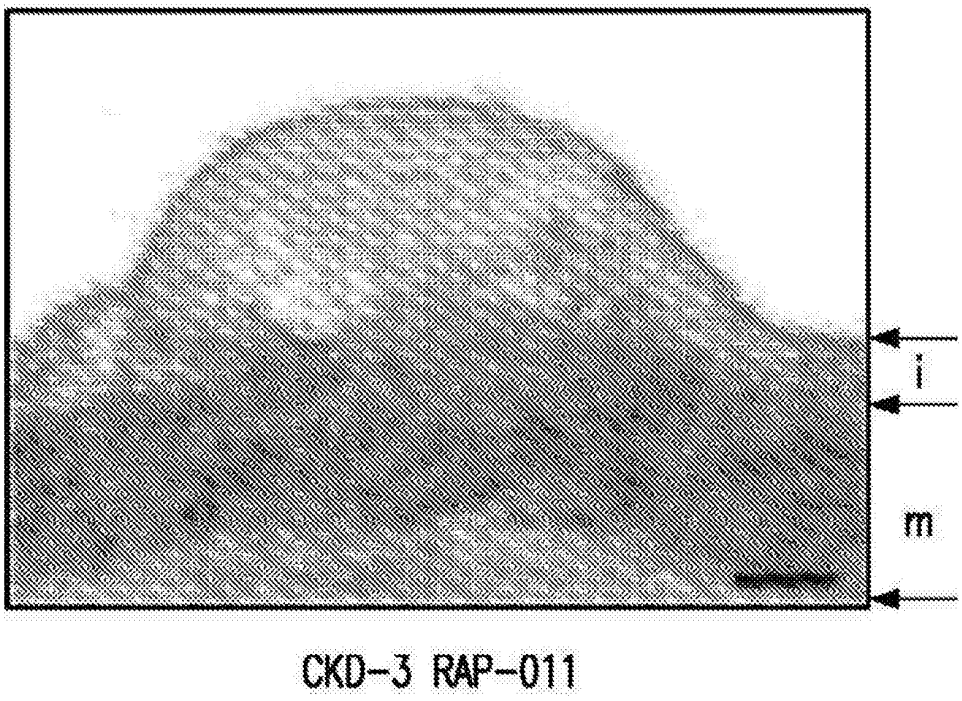


图26B

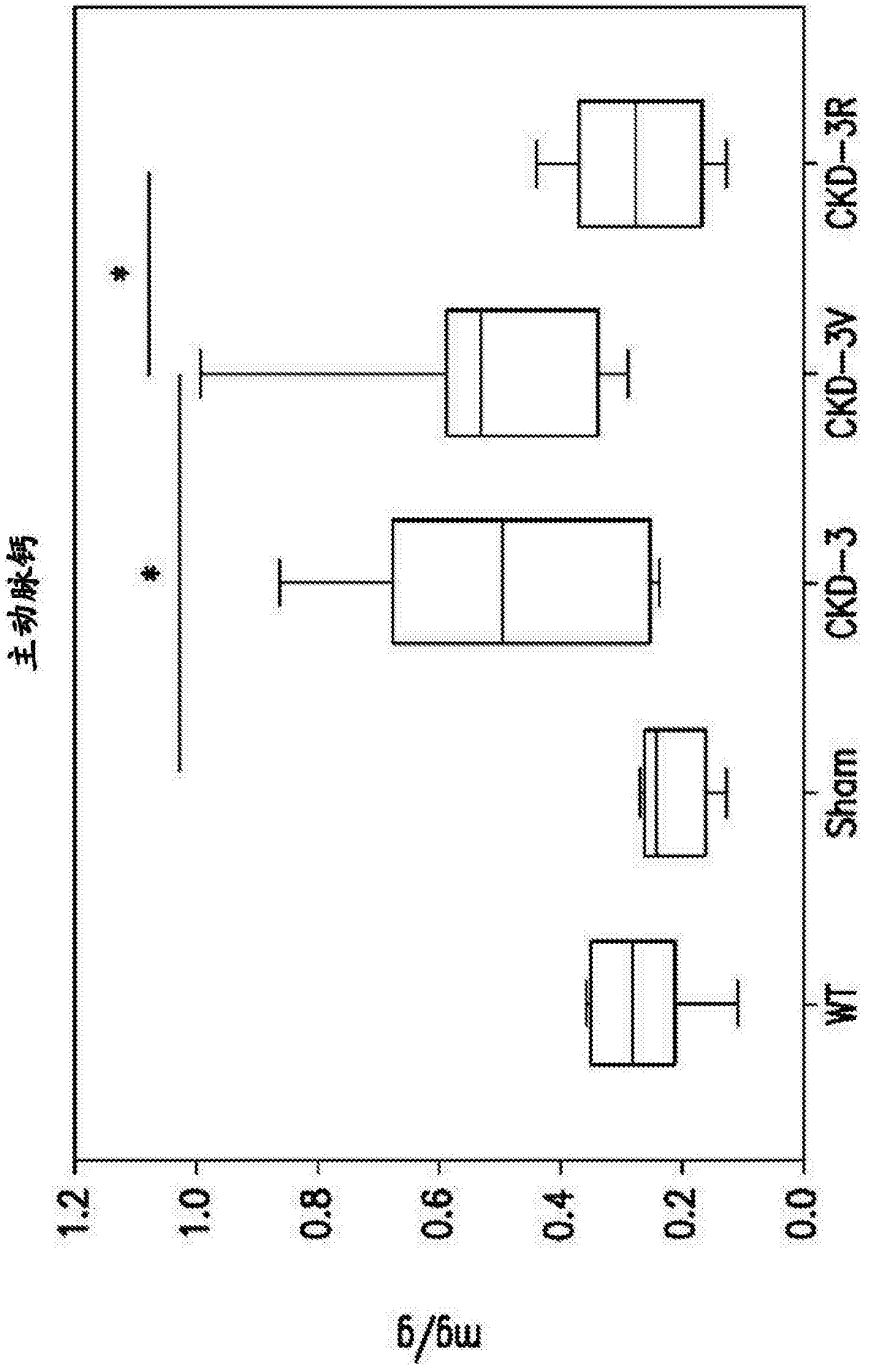


图26C

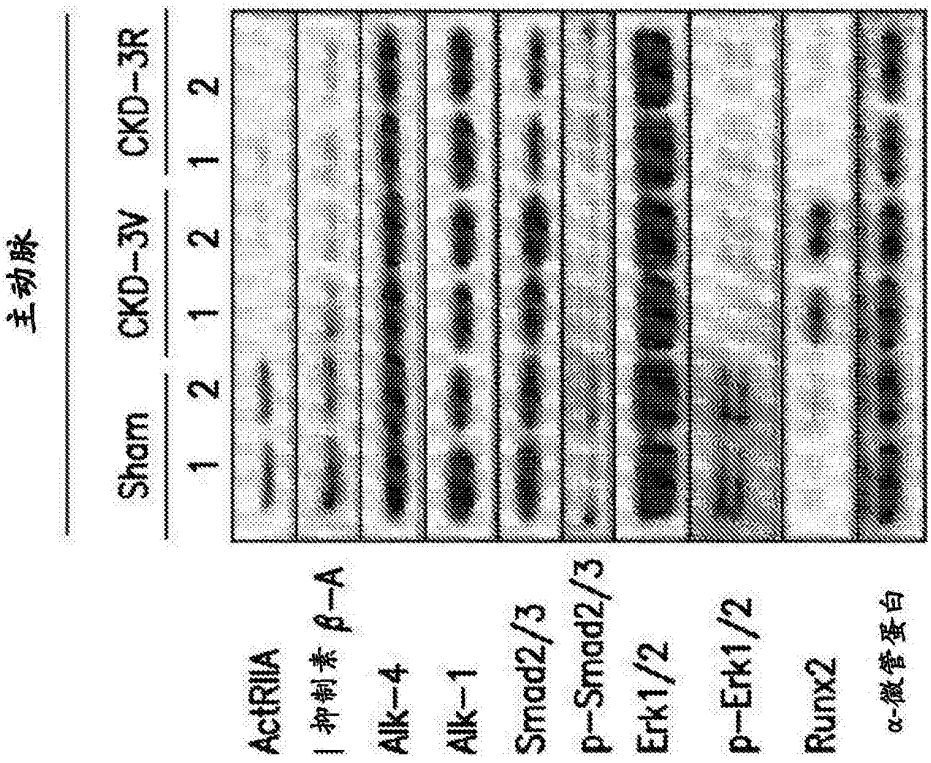

主动脉
Sham
CKD-3V
CKD-3R
1 2 1 2 1 2
ActRIIA
|-抑制素 β-A
Alk-4
Alk-1
Smad2/3
p-Smad2/3
Erk1/2
p-Erk1/2
Runx2
α-微管蛋白

图27A

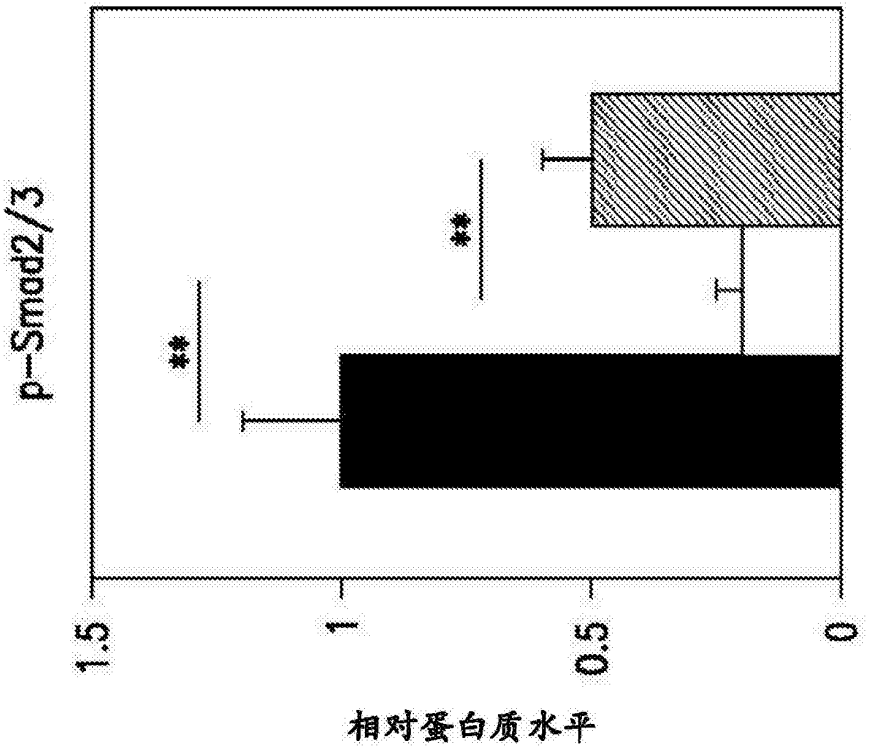

p-Smad2/3
**
**
1.5
1
0.5
0
相对蛋白质水平

图27B

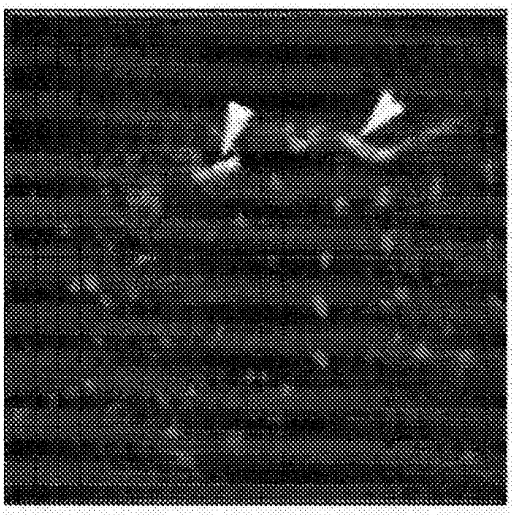


图28A

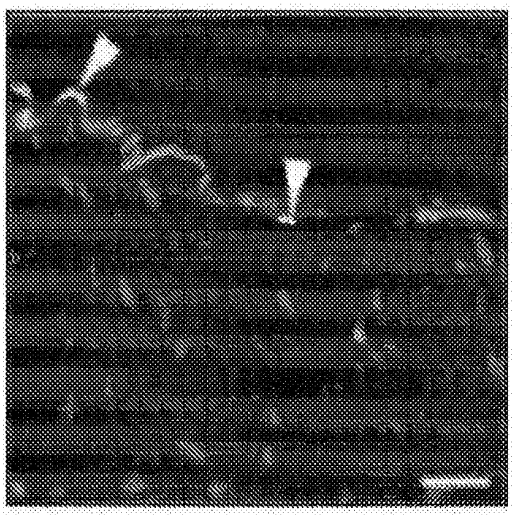


图28B

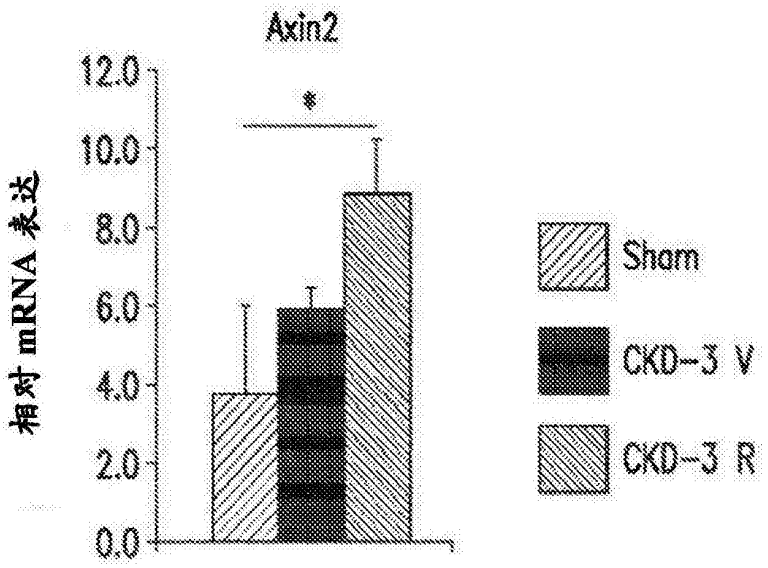

Axin2
*
12.0
10.0
8.0
6.0
4.0
2.0
0.0
相对 mRNA 表达
Sham
CKD-3 V
CKD-3 R

图28C

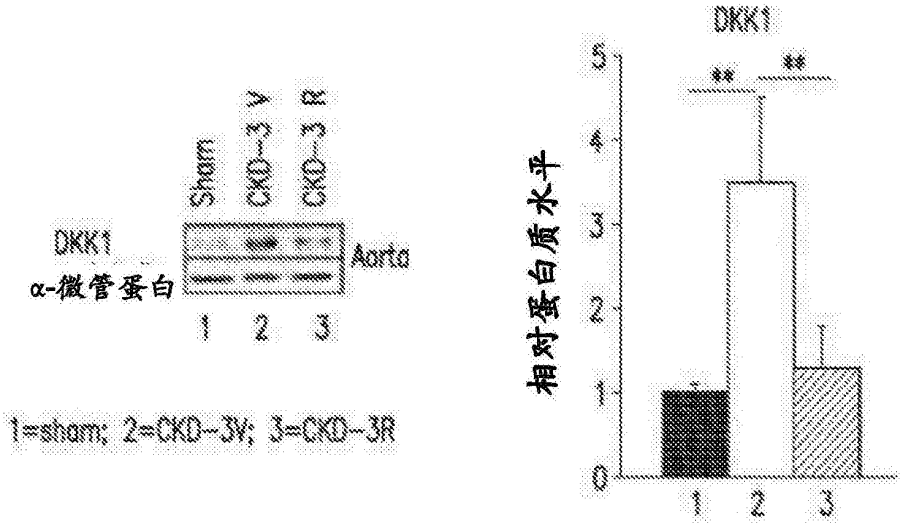

Sham
CKD-3 V
CKD-3 R
DKK1
α-微管蛋白
Aorta
1 2 3
1=sham; 2=CKD-3V; 3=CKD-3R
DKK1
**
**
相对蛋白质水平
5
4
3
2
1
0
1 2 3

图28D

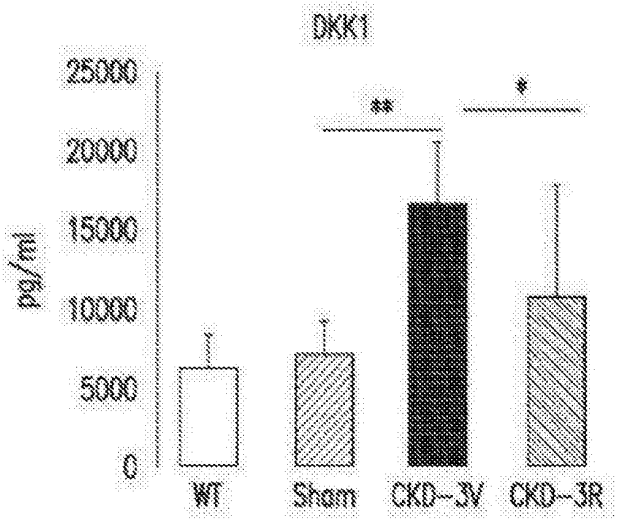

DKK1
25000
20000
15000
10000
5000
0
pg/ml
**
*
WT
Sham
CKD-3V
CKD-3R

图28E

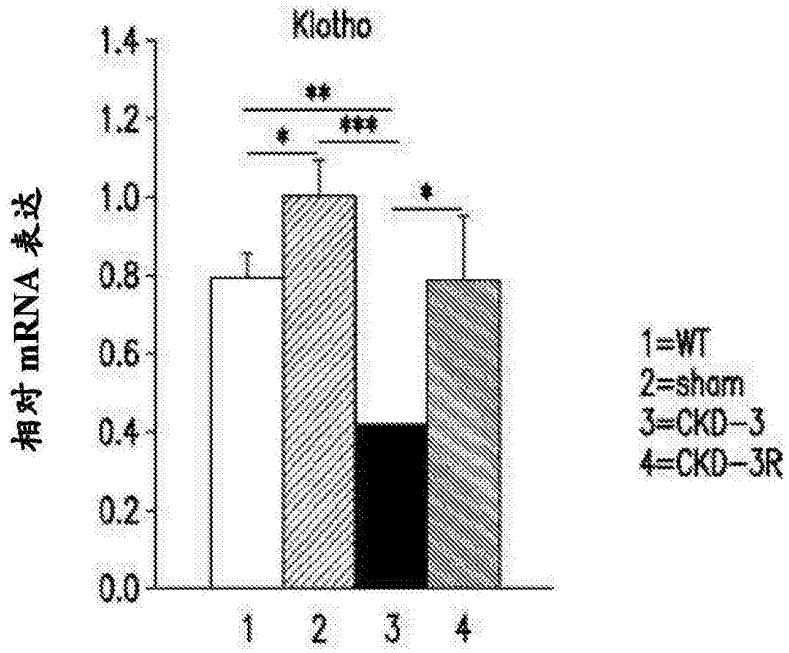

Klotho
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
相对 mRNA 表达
**
***
*
*
1 2 3 4
1=WT
2=sham
3=CKD-3
4=CKD-3R

图29A

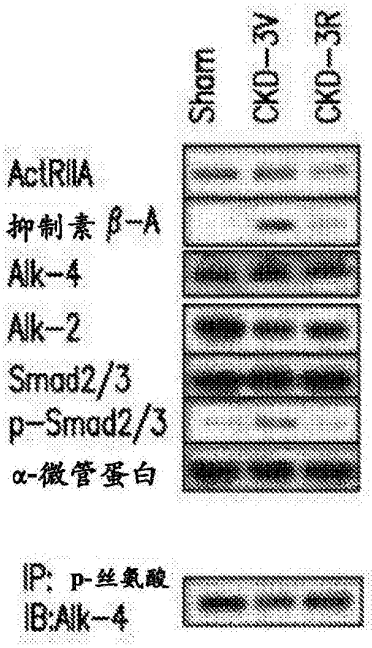

Sham
CKD-3V
CKD-3R
ActRIIA
抑制素 β-A
Alk-4
Alk-2
Smad2/3
p-Smad2/3
α-微管蛋白
IP: p-丝氨酸
IB:Alk-4

图29B

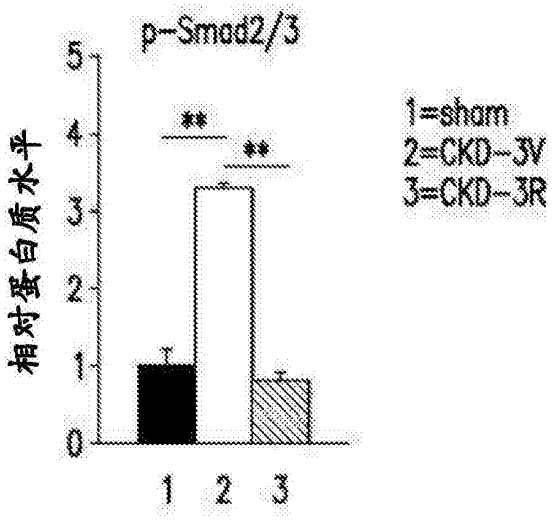

p-Smad2/3
相对蛋白质水平
0
1
2
3
4
5
**
**
1=sham
2=CKD-3V
3=CKD-3R
1
2
3

图29C

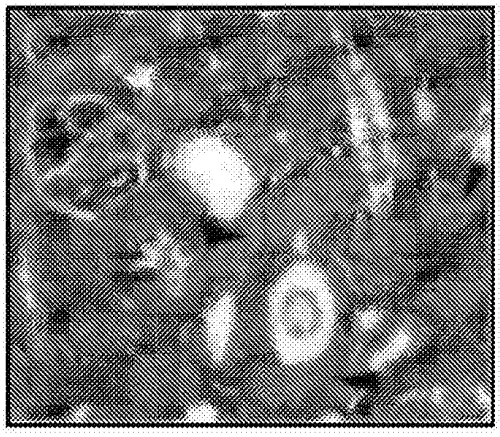


图30A

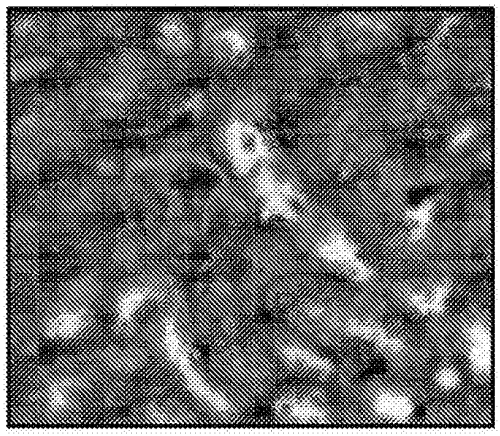


图30B

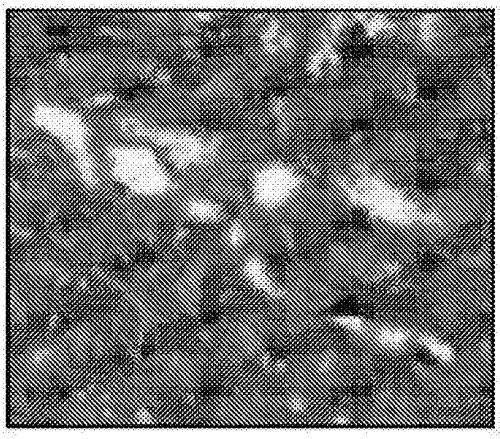


图30C

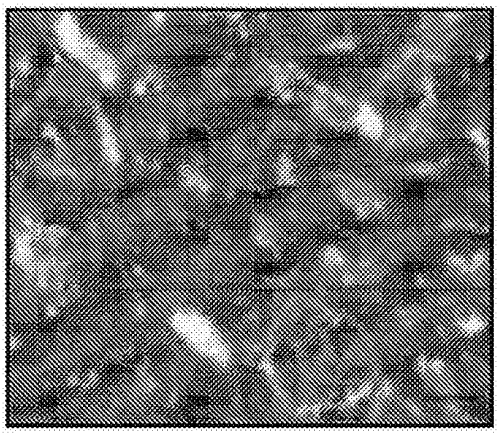


图30D

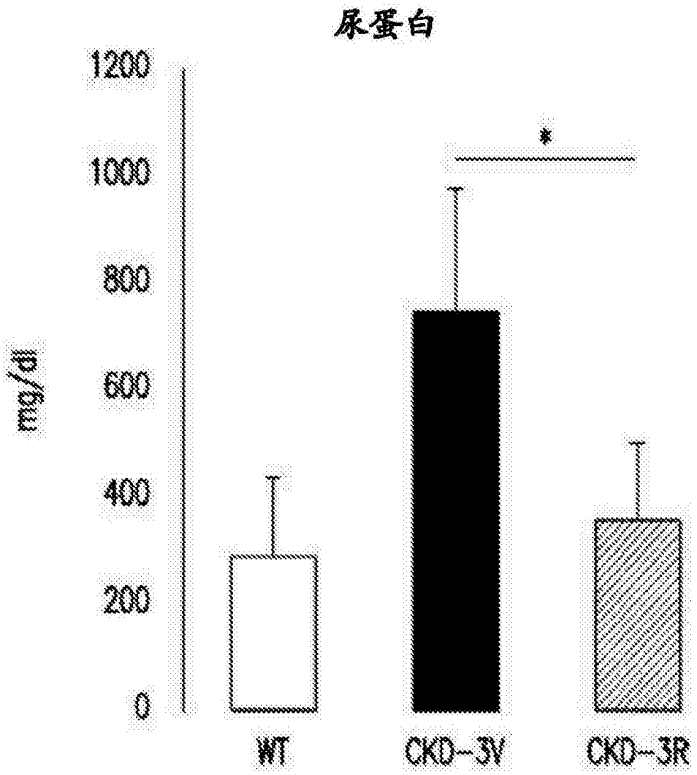


图30E

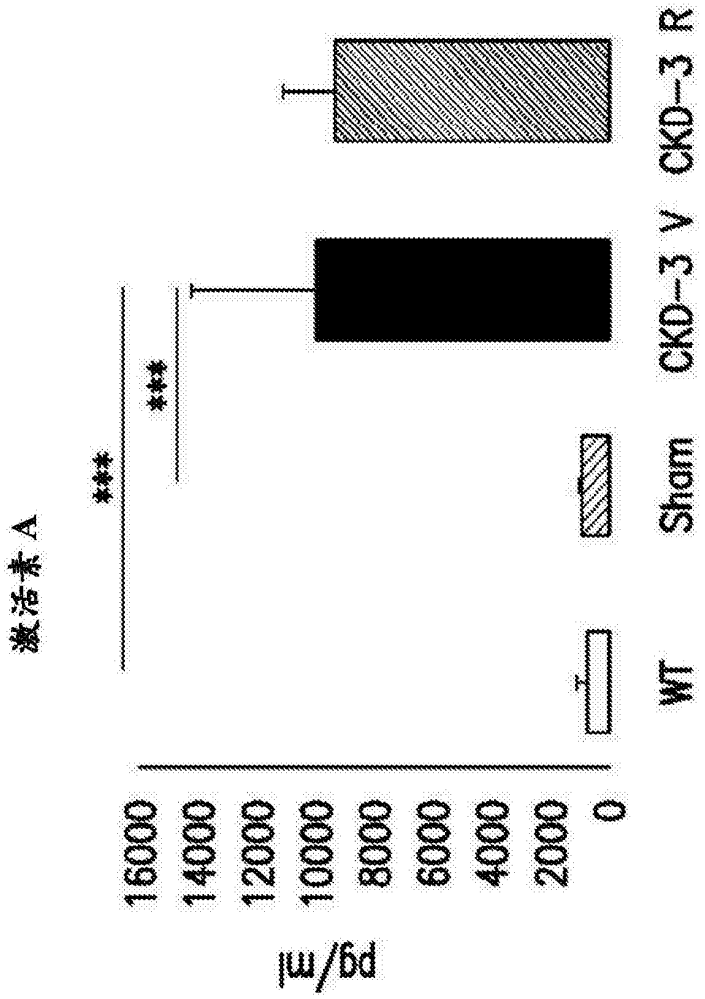

激活素A
pg/ml
16000
14000
12000
10000
8000
6000
4000
2000
0
WT
Sham
CKD-3 V
CKD-3 R
***
***

图31A

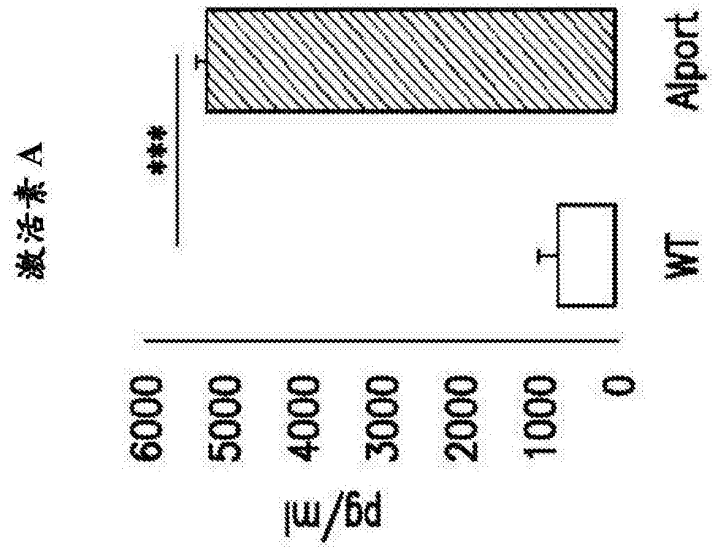

激活素 A
***
Alport
WT
6000
5000
4000
3000
2000
1000
0
pg/ml

图31B

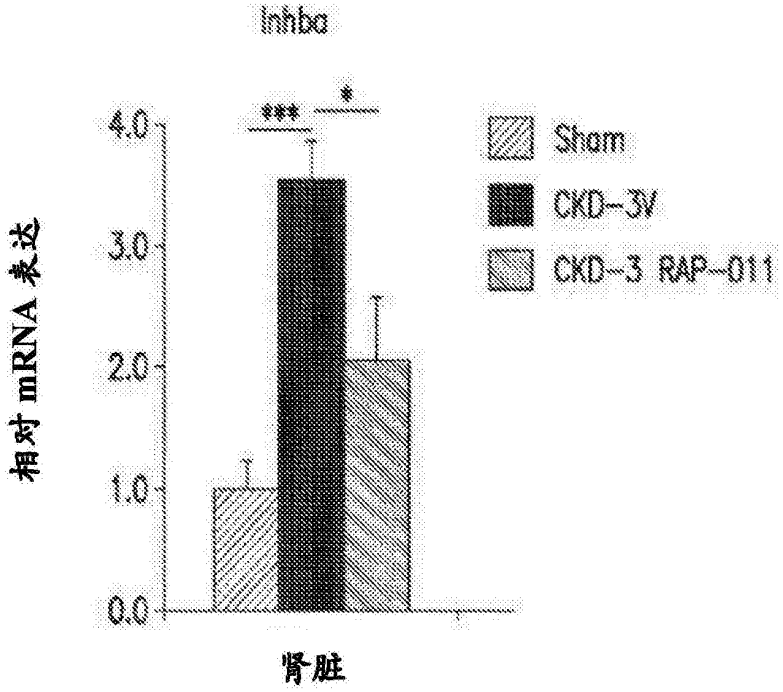

Inhba
***
*
4.0
3.0
2.0
1.0
0.0
相对 mRNA 表达
Sham
CKD-3V
CKD-3 RAP-011
肾脏

图31C



图31D

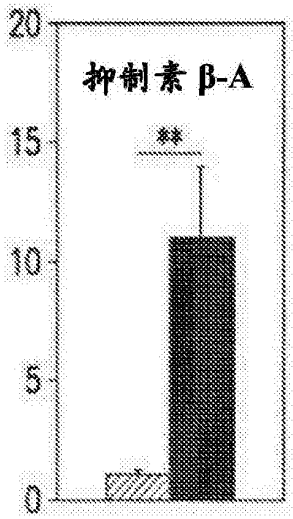


图31E

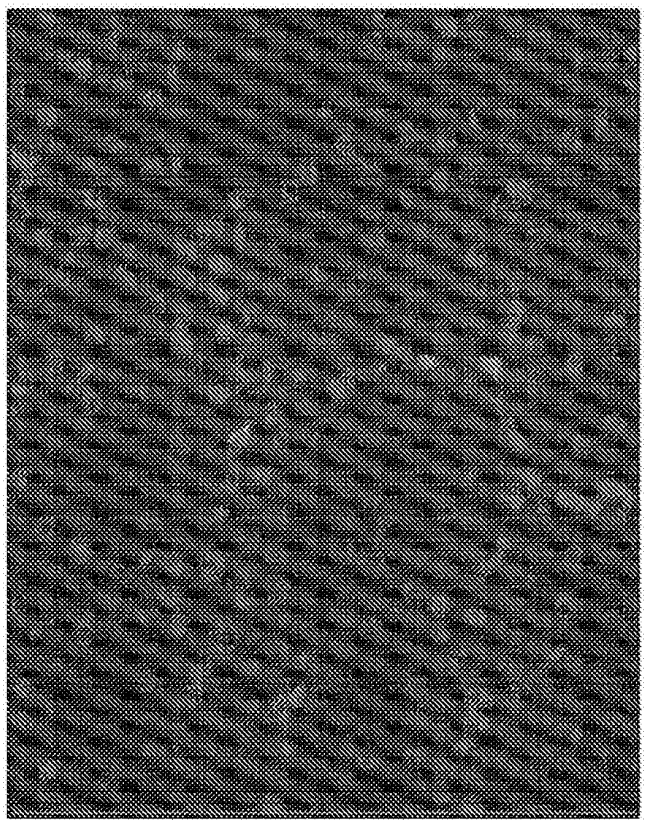


图32A

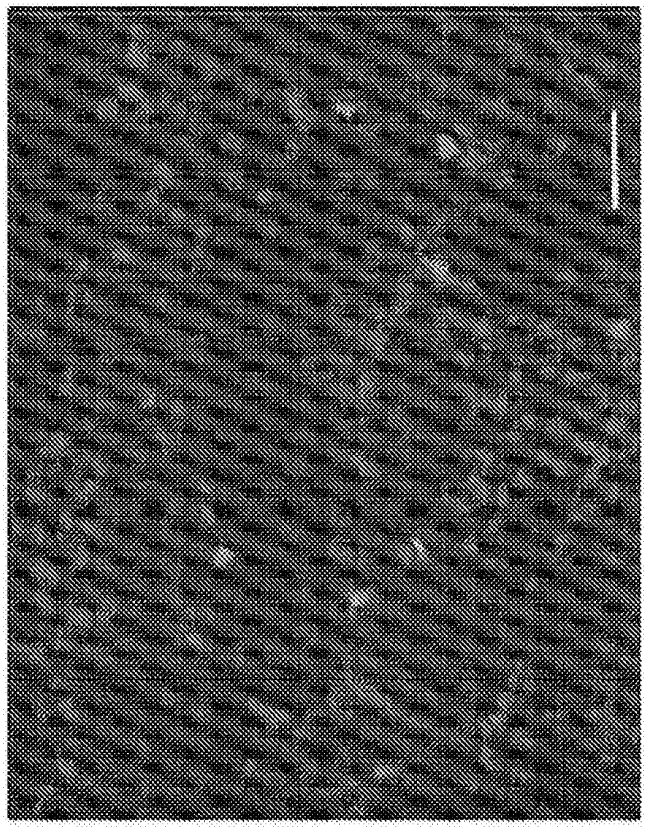


图32B

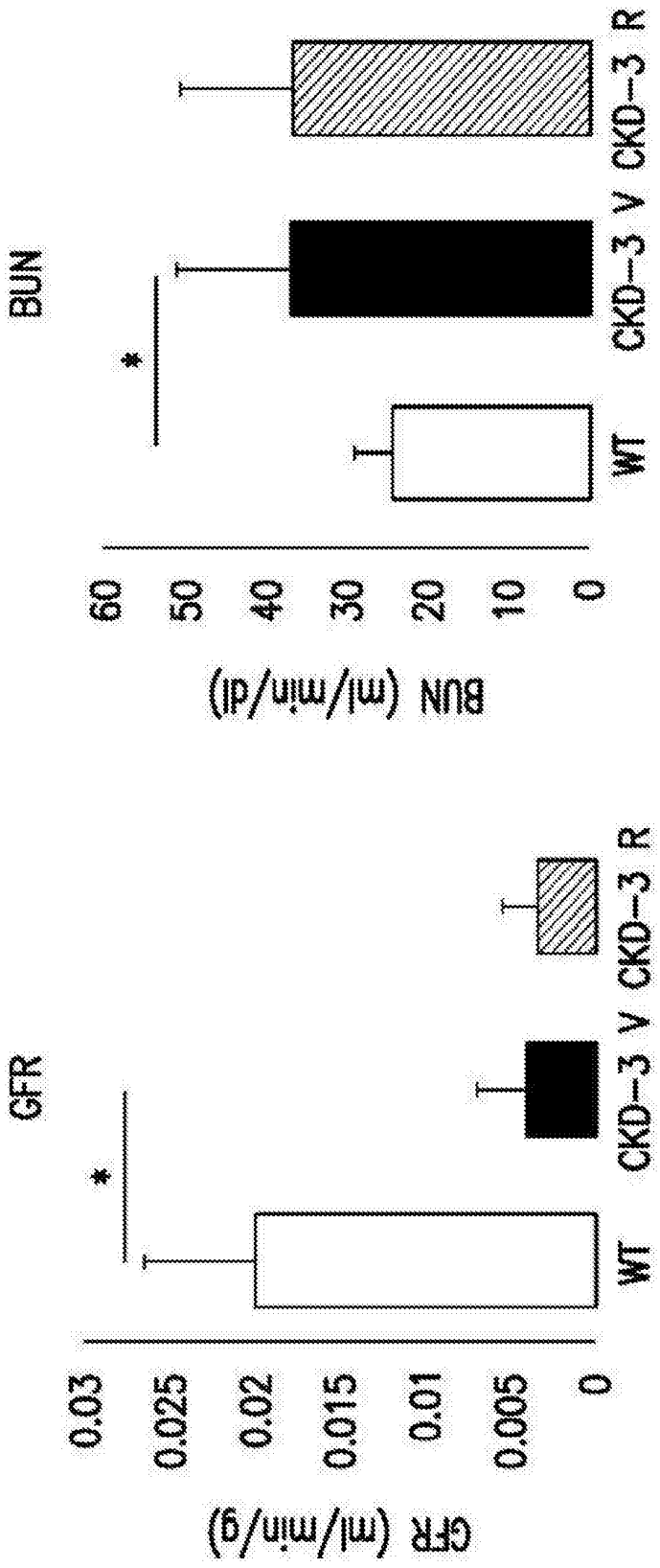
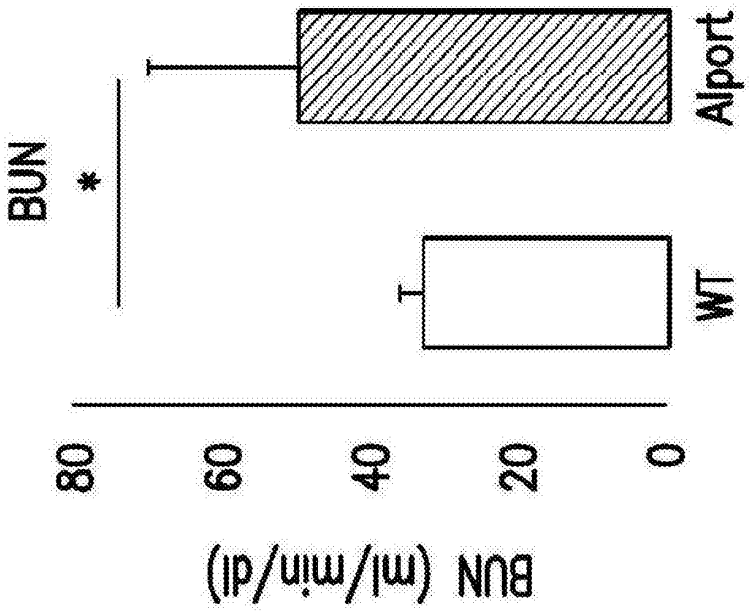

BUN
*
WT
CKD-3 V
CKD-3 R
60
50
40
30
20
10
0
BUN (ml/min/dl)
GFR
*
WT
CKD-3 V
CKD-3 R
0.03
0.025
0.02
0.015
0.01
0.005
0
GFR (ml/min/g)

图33A


BUN
*
Alport
WT
80
60
40
20
0
BUN (ml/min/dl)

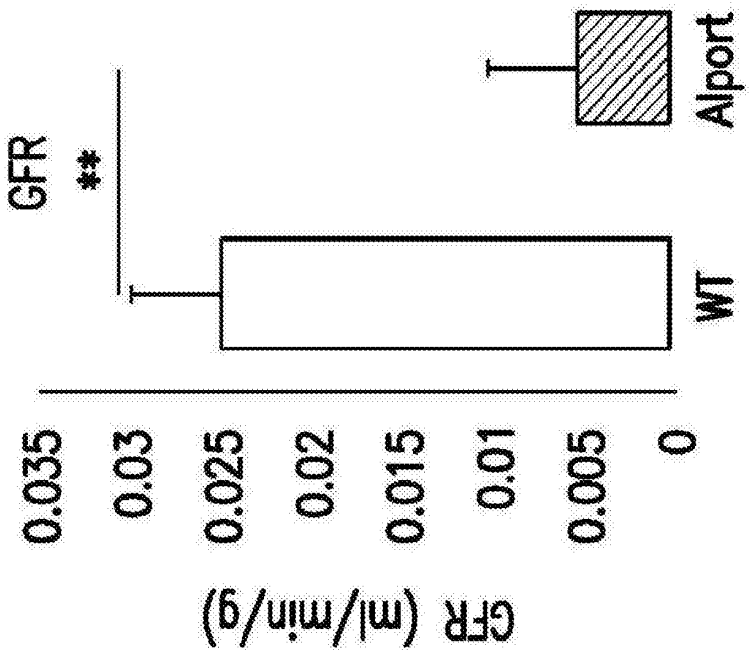

GFR
**
Alport
WT
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
GFR (ml/min/g)

图33B

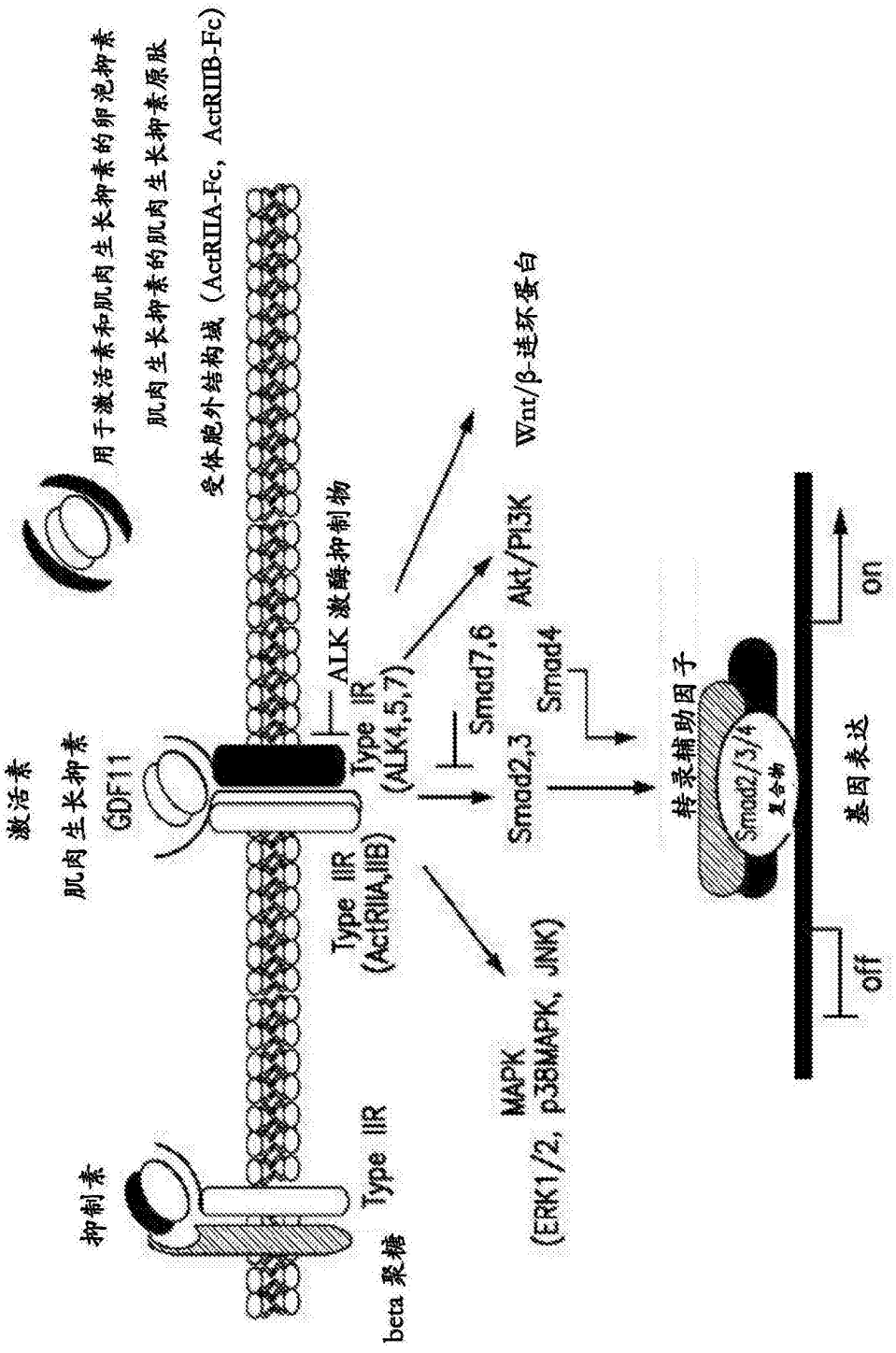

用于激活素和肌肉生长抑素的卵泡抑素
肌肉生长抑素的肌肉生长抑素原肽
受体胞外结构域（ActRIIA-Fc，ActRIIB-Fc）
激活素
肌肉生长抑素
GDF11
ALK 激酶抑制物
Type IR
(ALK4,5,7)
Smad7,6
Smad4
Akt/PI3K
Wnt/β-连环蛋白
Smad2,3
转录辅助因子
Smad2/3/4
复合物
on
基因表达
off
Type IIR
(ActRIIA,IIB)
抑制素
Type IIR
beta 聚糖
MAPK
(ERK1/2, p38MAPK, JNK)

图34



图35A

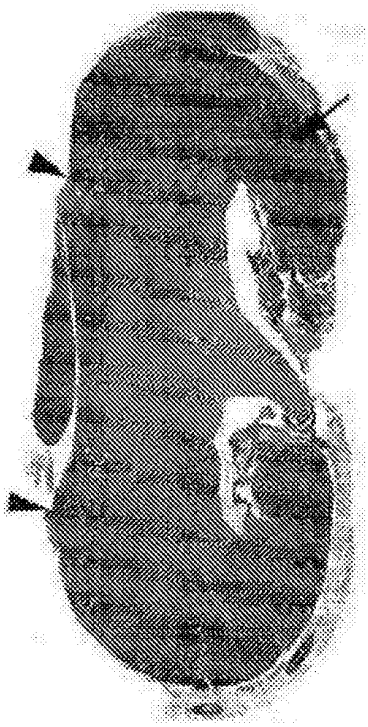


图35B



图35C



图35D

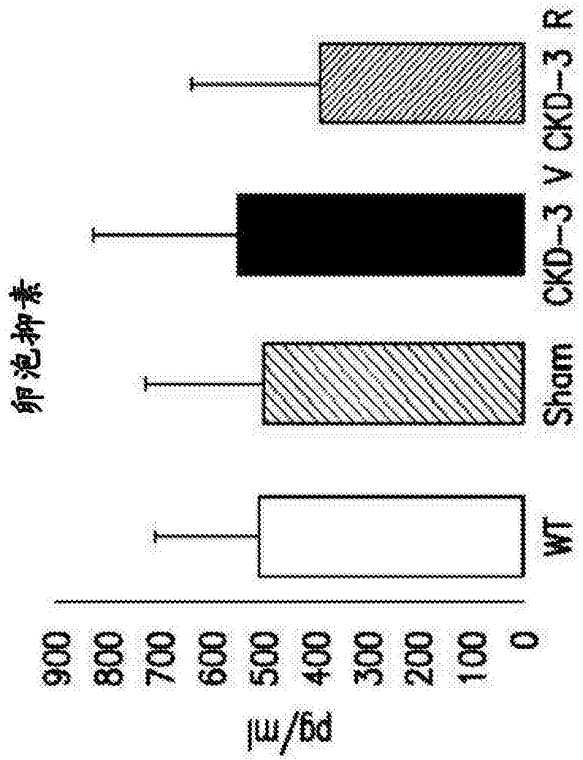

卵泡抑素
WT
Sham
CKD-3 V
CKD-3 R
0
100
200
300
400
500
600
700
800
900
pg/ml

图36A

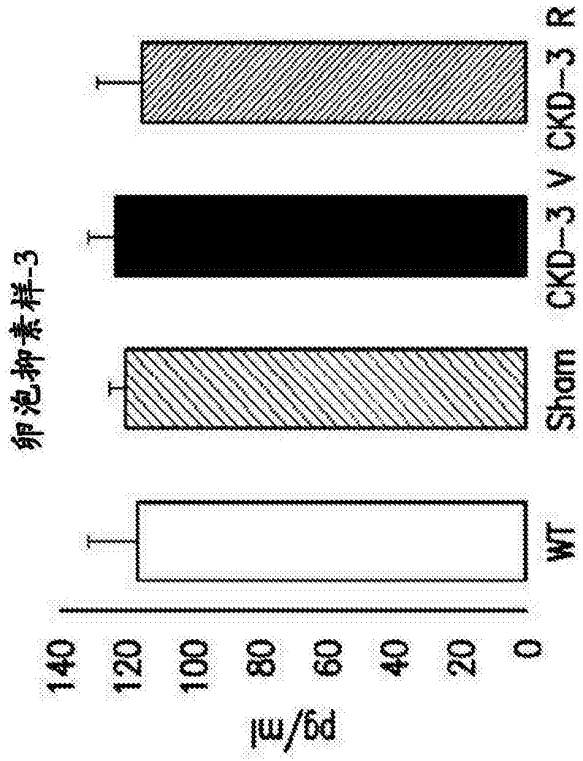

卵泡抑素样-3
WT
Sham
CKD-3 V
CKD-3 R
0
20
40
60
80
100
120
140
pg/ml

图36B

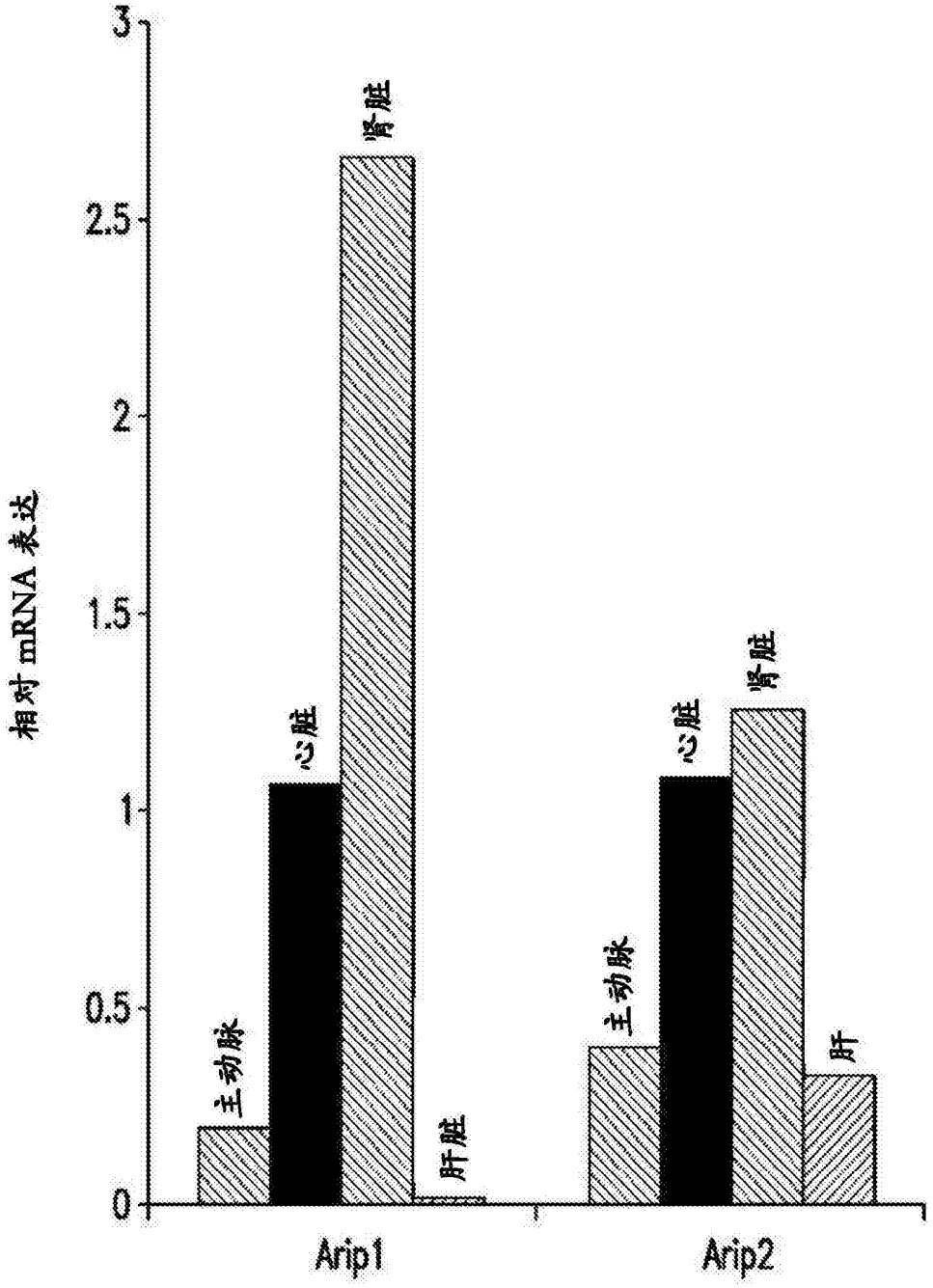


图37